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# FUSARIUM-WILT OF TOBACCO[1]

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## INTRODUCTION

During the summer of 1916 the writer's attention was called to a wilt disease of tobacco occurring near Benedict, Charles Co., Md. The disease occurred on the Maryland Broadleaf variety of tobacco, which was nearing maturity, and showed all the appearances of a typical wilt disease. Plants in all stages of wilting were found, from those showing the first signs of infection to those in which all the tissues of the plant were dead. When the stalks or midribs of the leaves were cut, the fibrovascular bundles were found to have a distinctly brown to black color in place of the normal white. It was at first suspected that the bacterial wilt due to *Bacillus solanacearum* Erw. Smith had been introduced into the Maryland tobacco fields. Although the general symptoms of the disease were very similar to those of bacterial wilt, the absence of bacterial ooze, the uniform occurrence of Fusarium on plated out material, the absence of vessels filled with bacteria, and the presence of fungus strands in the vessels gave strong evidence that bacteria were not concerned. Considerable difficulty was at first encountered in getting good infection with the Fusarium isolated. When artificial infection was finally secured, however, further study of this disease became of special interest, since no Fusarium-wilt disease of tobacco has apparently been proved to exist, although, as will be shown, in one case it seemingly had been reported erroneously, and in another case a Fusarium disease, apparently not a wilt, has been described. The present paper is intended primarily to establish the occurrence of a Fusarium-wilt of tobacco, with a description of the causal organism and a discussion of certain matters bearing on the control of the disease under practical conditions.

[1] Cooperative investigations of the Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

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## OCCURRENCE OF THE DISEASE

In the summer of 1916 the disease was found only on the plantation of Mr. James H. Bolling near Benedict, Charles Co., Md. It was serious in only one field of about 6 acres on this farm, where perhaps 10 to 20 per cent of the plants were dead or showed symptoms of the disease, although in smaller areas in the field it is estimated that 50 to 75 per cent of the plants were damaged (Pl. 63, A). According to Mr. Bolling and the tenant on the farm this disease had occurred at intervals for many years on this farm but not so seriously as in 1916.

During the summer of 1917 Charles County was again visted, with the result that the disease was found on two other farms near Newport, Md. The disease was not apparently so serious this season as in the previous one. This region was not visited during the seasons of 1918 and 1919, and nothing further is known of the disease in that section.

In the summer of 1919 a "new" disease of tobacco was called to the writer's attention by correspondence from Clermont Co., Ohio, and specimens were received through the courtesy of Mr. David Geesner of Owensville on September 20, which showed typical symptoms of Fusarium-wilt on mature plants of the White Burley variety. Sixty-six pieces from diseased portions were plated out, practically all of which yielded Fusarium, from which artificial infection was later secured. The disease is also said to have occurred previously in the vicinity of Owensville.

The symptoms of the disease are so evident that growers could not fail to note and report its occurrence. On account of the scarcity of such reports either from the farmers or experiment station workers in the tobacco-growing regions outside of the Granville (bacterial) wilt areas it is believed that the Fusarium-wilt is not a serious disease and probably will never become of great economic importance. If, however, it becomes more generally introduced into the White Burley districts it may become a serious parasite, since this variety, as will be shown, is very susceptible to the wilt.[1] In North and South Carolina, Georgia, and Florida where the Granville wilt occurs, it is possible that the Fusarium-wilt is also present, but growers as well as plant pathologists would be likely to report such cases as Granville wilt unless a special examination of the diseased tissue were made. It is not believed that there is much danger that this disease will become serious in the northern cigar tobacco growing regions on account of the resistance of the varieties grown and the climatic conditions prevailing.

## REVIEW OF THE LITERATURE

The occurrence of Fusarium-wilt diseases of a considerable number of plants are now reported in literature. The Fusarium-wilt of tobacco possesses much in common with these diseases in that it is a vascular disease. However, it is not proposed here to enter into a review and

[1] During the summer of 1920 specimens of Fusarium-wilt were received from the White Burley district of Kentucky.

comparison of these diseases. The Fusarium problem viewed as a whole or even as that part which has to do with the nomenclature of the vascular parasites, is recognized as being in a rather unsatisfactory state. Rather uncertain precedent in naming forms, together with the plasticity in physiology, and, one is tempted to say, in morphology of the forms themselves, is the cause of the greatest difficulties encountered in this problem. It is felt, therefore, that until a more detailed study of the Fusaria causing wilt of tobacco and related plants can be made, it will not be profitable to enter upon a review preliminary to discussion of this subject. The review here presented, therefore, includes only the evidence which we now have relating to Fusarium as a probable cause of disease in the tobacco plant.

McKenney (7)[1] in 1903 described a wilt disease of tobacco in North Carolina as due to Fusarium. No proof of pathogenicity was obtained. This disease was soon afterward studied by Stevens and Sackett (*11*) and by Smith (*10, p. 220–271*) and was found to be a bacterial wilt (*Bacillus solanacearum*), so that Fusarium could no longer be associated with the disease. According to Smith no good evidence for a Fusarium-wilt existed; but, reasoning from the universal distribution of Fusarium and its occurrence as a vascular parasite in plants closely related to tobacco, he predicted that a Fusarium-wilt of tobacco would be found. Judging from the description of McKenney's disease and the virulence attributed to it, the writer believes it could not have been Fusarium-wilt.

Lounsbury (*6*) in 1906 reports a wilt disease of tobacco in the Kat River Valley, Cape of Good Hope, which he states is, in his opinion, not similar to the American (Granville) wilt. Bacteria, fungi, and insects are all said to be concerned. Smith (*10, p. 220–271*) places it as a doubtful bacterial wilt. To judge from the description, this may have been a Fusarium-wilt disease, at least the South African disease should again be checked up, if it still occurs.

Petch (*9*) in 1907 reported a disease of tobacco in Dumbara, Ceylon, which is said to be a "root-disease" causing "sudden and premature ripening," killing out plants in patches. The stem is said to be discolored at the base. A Fusarium was isolated from the roots. This description may fit one or more diseases of tobacco. The isolation of a Fusarium from the roots is, of course, of no significance. The "sudden and premature ripening in patches," however, suggests a wilt disease.

Delacroix (*2*) in 1906 reported a disease of tobacco occurring around Perigneux and Razoc, France, as due to a species of Fusarium which he named *Fusarium tabacivorum*. The disease is said to resemble superficially a bacterial cancer localized at the collar of the plant, and the port of entry of the parasite is believed to be always an insect puncture. The mycelium of the fungus was found to be present throughout the whole

[1] Reference is made by number (italic) to "Literature cited," p. 534–535.

base of the stalk when the disease was well established. The fungus is said to lose its virulence in culture after the "first generation." The conidia are described as straight or slightly curved, round obtuse at both extremities, possessing usually three septa, their size varying from 25 to 35 microns by 4 to 6 microns.

Delacroix's Fusarium disease is probably not a true wilt disease, since it is not described as such. The description and illustration of the causal organism are, furthermore, too fragmentary and unsatisfactory to permit of comparison. The new species created (*Fusarium tabacivorum* Delac.) has apparently not been credited by any recent workers with the Fusaria. It is interesting to note that Delacroix knew of McKenney's Fusarium disease but could not say whether his disease was identical with it or not. In view of the fact that Delacroix's description may fit other diseases of tobacco as far as symptoms are concerned, and since we have only the statement that infection has been secured with an organism of such universal occurrence as Fusarium, together with the unreliable description of the causal organism, it is difficult to see how at the present time we can accept either the disease as such or the species described as authentic.

A brief abstract was published by the writer in 1918 (*4*) calling attention to the wilt disease in Maryland and giving reasons for believing it was due to Fusarium, although artificial infection had not been secured at that time.

## SYMPTOMS OF THE DISEASE

The symptoms of the disease may first become evident upon very young or on nearly mature plants. Under the field conditions observed it is evident that plants may succumb at any stage in their growth, although it is not clear as to what time the original infection of the plant occurs. It seems probable that infection may take place at any time, but that it is more likely to occur when the plants are young, the parasite remaining in a more or less latent stage until favorable environmental conditions for the further development of disease occur. In full-grown plants the earliest symptoms seem to be the sudden wilting of only one or more leaves on the plant, accompanied by yellowing and finally browning and death, but not decay of the leaf. In some cases this symptom at first may be localized on only one side of the leaf. At other times all the leaves in a narrow vertical band, comprising about one-fourth or one-eighth of the leaves of the plant, may become wilted while the others remain apparently free from the disease (Pl. 64, A). If the stalks of such plants are cut, it will be found that the discolored bundles are confined to only a part of the circumference of the vascular ring. All degrees of wilting from those described, to complete collapse of all the leaves on the plant, however, may occur (Pl. 64, B). If the plants are pulled up, large or small dead roots may be found, while others are appar-

ently healthy. If, now, the diseased stalk, roots, suckers, midribs, or veins of the leaves are cut either in cross section or longitudinally (Pl. 65, B) the vascular system will be found to be brown or distinctly black, but upon pressure no "ooze" appears. The vascular decay is distinctly "dry."

On young plants in the greenhouse where the writer has had an opportunity to note the symptoms of the disease more carefully they are essentially the same so far as the vascular system is concerned, but the leaves first lose their chlorophyll, becoming yellow and somewhat wrinkled but distinctly turgid and "brittle," as compared with healthy leaves. This condition may obtain for some time previous to wilting unless exceptionally high transpiration occurs. The leaves, of course, finally dry up as they do in the field (Pl. 63, C). In the greenhouse the symptoms are most likely to appear first on the youngest leaves, and this may be more or less characteristic in the field.

So far as has been noted the parasite is not able to cause any rotting of the living parenchymatous tissues of the plant. In heavily infested soil where the cortical layers of the plant have been severely wounded or a leaf petiole has been broken off below the surface of the soil, the parasite may enter the vascular system readily and cause the death of the aerial portion without in any way affecting the parenchyma of the stem or roots at or below the surface of the soil.

Histological studies of the disease were made by various methods, but best results were secured by killing and fixing young tissue in Gilson's fixative, imbedding in paraffin, and staining with the Pianese stain, as described by Vaughan (12). Transverse sections of infected stems or midribs of leaves (Pl. 66, A) showed that all the vessels in local areas of the vascular ring were more or less invaded, sometimes almost completely "clogged" with mycelium. Longitudinal sections (Pl. 66, B) showed in an even more striking manner the general occurrence and the "bunching" of mycelium in the vessels. Nevertheless, from the behavior of the diseased plants, especially with regard to yellowing and early turgidity, it is not believed that death of the plants is due to clogging of the vessels but rather to toxic materials formed by the parasite or as a result of the parasitic action on the host.

## ISOLATION AND INFECTION EXPERIMENTS

In the first isolations pieces of the discolored portions of the stem, together with some surrounding healthy tissue, were cut out and treated with 1 to 1,000 mercuric chlorid for 30 to 120 seconds, rinsed in sterile water, and placed on hard potato agar in Petri dishes. Growth of fungus mycelium from the diseased tissue was slow and not uniform. Pure cultures of Fusarium, however, were secured. Isolations were later made by cutting off the cortical layers with a hot blade and cutting out

fairly large pieces under as sterile conditions as possible, rinsing these through 5 to 10 sterile water blanks, transferring to a sterile Petri dish, where they were further cut up into small pieces and transferred to 10 cc. of potato agar in a Petri dish acidified with two to three drops of 25 per cent lactic acid. Out of hundreds of pieces plated out in this manner apparently pure cultures of the causal organism were rapidly secured in practically all cases. Mercuric chlorid treatment apparently resulted in part of the chlorid entering the bundles, from which it was not readily washed out, and consequently did not prove useful for plating out in this case.

Single spore isolations were made from the Maryland Fusarium, and these have been used in some but not in all infection experiments, cultural studies, and spore measurements.

Infection experiments during the summer of 1917 consisted chiefly in inoculating large plants in the field with pure cultures of the Fusarium through wounds in the stalk. No infection was secured except in one instance which was questionable. In the fall of 1917 sterilized soil was inoculated with mycelium from pure cultures of the wilt Fusarium, and very young White Burley tobacco plants were transplanted into it. After about five weeks several of the plants wilted and died. Infection thereafter was intermittently secured on White Burley through the medium of the soil. The inoculum was usually grown on a mixture of 100 gm. of sand, 10 gm. of corn meal, and about 1 gm. of glucose to 50 cc. of water in 1-pint milk bottles or mason jars. This culture medium was cooked for one hour in the autoclave, then stirred up so as to render the medium "spongy," and again sterilized. After being cooled, the medium was inoculated with the Fusarium and incubated at 25° to 30° C. for four or five weeks, after which the inoculum was allowed to dry sufficiently to permit pulverizing, when it was thoroughly mixed with the soil. Good infection was also secured from mycelium and spores directly from potato agar tubes and also from a suspension of conidia alone. The latter method was usually not so successful as the former. Failure to secure as high precentage of infection at one time as at another led to a preliminary study of environmental and other conditions favoring the disease, and these will be reported upon briefly in this paper. It should be stated here, however, that as soon as the plants were intentionally wounded more uniform results in infection were secured. Ordinarily this consisted simply in pulling or pinching off one or two of the lower leaves and setting the plant deep enough to bring the resulting wound below the surface of the soil. Although it can not be said with certainty that infection would never occur in a plant perfectly free from wounds of any sort on the root or stem, it is quite certain that infection is greatly enhanced by wounding. The first signs of infection on leaves have been secured in as short a time as two days after exposing wounded stems to heavily infested soil.

## CAUSAL ORGANISM

The causal organism can be readily isolated from diseased tissue by plating out on acid potato agar. The mycelium ordinarily imparts only a dull pinkish tinge to the substratum and seemingly has a more or less characteristically sparse growth and "powdery" surface (Pl. 65, A) as compared with the dense cottony development of some forms of Fusarium. The "powdery" appearance is due to microconidia which are formed in abundance, as is characteristic on a number of other media where similar growth is made. "Strains" bearing sporodochia may or may not occur. Where fruiting "strains" have been secured sporodochia have usually been produced in abundance, especially on Melilotus stems, oatmeal agar, and occasionally on potato agar and other media. True

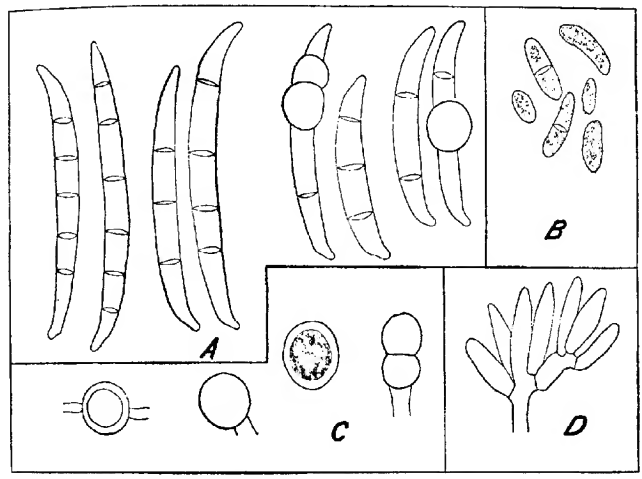


FIG. 1.—Camera-lucida drawings of spore forms of *Fusarium oxysporum* var. *nicotianae*, n. var: A, macroconidia; B, microconidia; C, chlamydospores; D, conidiophore of the sporodochial stage.

pionnotes have not been observed in the cultures during a period of four years on various kinds of media. "Pseudopionnotes" or reduced pionnotes could, however, be made to appear. Blue sclerotia and sometimes salmon-colored sclerotia are produced.

An examination of the conidia from well-developed sporodochia of the Maryland strain ordinarily shows a preponderance of 3-septate conidia, which, together with the shape and size of the spores (fig. 1) and the fact that the fungus produces a wilt disease, placed it readily in the section Elegans, according to Wollenweber's classification (*13*). A more careful study of the size and shape of the conidia brings out a close resemblance to *Fusarium oxysporum* Schlecht., according to recent descriptions of this species. Studies were therefore undertaken to establish whether the tobacco-wilt Fusarium is identical with *Fusarium oxysporum*

as described. After the conclusion was reached that the tobacco-wilt Fusarium is related morphologically to *Fusarium oxysporum* but is not identical with it, several methods of study were undertaken with the hope of furnishing further evidence. These consisted of (1) infection experiments with the tobacco-wilt Fusarium on the potato and certain other plants, (2) comparative cultural studies with *Fusarium oxysporum* strains secured from other sources, and (3) infection experiments with strains of *Fusarium oxysporum* from potato upon tobacco.

Several attempts at producing infection with the Maryland strain of the tobacco-wilt Fusarium on the potato vine failed. Potatoes were grown in artificially infested soil, and in several instances the stems were wounded immediately below the surface of the soil. This, however, is not regarded as conclusive evidence that infection is unobtainable.

Authentic cultures of *Fusarium oxysporum* were sought from various recent workers on this species. Cultures of Dr. Wollenweber's strain (No. 207) were received through Dr. W. A. Orton, and also a strain (No. 208) isolated by Dr. H. A. Edson from potatoes. From Minnesota, Bisby's culture No. 3315 and a reisolation from inoculation on potato were secured (*1*). From the stock cultures in the Department of Plant Pathology at the University of Wisconsin two strains were received, numbered 226 and 227, both apparently originally from Dr. Wollenweber to Link at Nebraska and thence to Goss at Michigan, who brought them to Wisconsin. Finally a culture of MacMillan's strain (*8*) from potatoes in Colorado, which was sent by MacMillan to the Department of Plant Pathology at Wisconsin, was obtained. None of these cultures was apparently in a good normal growing condition when transferred to my media, as compared with more recently isolated forms on the same media. The growth may be best expressed as "slimy" in nature, as if bacterial contamination had occurred—that is, aerial growth was sparse or absent and a rather thin mycelial growth was formed on the surface of the media only. Many microconidia and some macroconidia were produced. Repeated trials on various media failed to bring about the sporodochial fruiting stage, without which a satisfactory comparison with the septate conidia of the tobacco-wilt Fusarium could not be made. Therefore, the cultures were at first used largely for comparison of cultural characteristics on different media, especially on Melilotus stems, cooked rice, oatmeal agar, potato plugs, and potato agar. The various strains of *Fusarium oxysporum* from the various sources did not behave in a similar manner on the same media, and consequently it was felt that the significance of the cultural comparisons obtained was much reduced. Whether this condition was due to differences in age or condition of the strains or to actual physiological differences inherent in the strains can not be said.

The following notes were taken on the growth of the tobacco-wilt Fusarium on various media in an early trial. Not much emphasis can be

placed on the shade of the pigment given, because comparisons were not made with Ridgeway's color standards and nomenclature at this time.

ACID POTATO AGAR.—Good but rather light and "fluffy" aerial growth, pure white, no agar coloration to a pale pink coloration, and formation of blue-green sclerotial masses at margin of agar in older cultures.

POTATO PLUG.—Excellent growth, mycelium becoming faintly salmon-colored and plug deep blue in parts; abundant formation of small bluish black sclerotia in older cultures.

OATMEAL AGAR.—Good growth, pale salmon-colored mycelium, medium changing to pale lilac. Large sclerotial masses form at base of agar.

RICE.—Good growth of white to pink mycelium.

MELILOTUS STEM.—Fair growth of white to pink mycelium. Sporodochia formed abundantly after 15 to 30 days. Sporodochia forming singly or in large masses. Pale to deep salmon in color. Abundant production of small bluish black sclerotia.

STRING-BEAN PLUG.—Excellent growth with production of lilac-colored mycelium.

CARROT PLUG.—Good growth with faint lilac coloration.

LIMA-BEAN AGAR.—Fair to poor growth only, hardly any pigment production.

CORN-MEAL AGAR.—Poor growth, practically no pigment production.

SYNTHETIC AGAR.—Good growth of white mycelium.

GELATIN (BEEF).—Fair growth with some liquefaction.

TOBACCO AGAR (FROM GREEN LEAVES).—Very poor growth.

The cultural differences between the various strains of *Fusarium oxysporum* used and those of the tobacco-wilt Fusarium are not believed to be of sufficient importance to warrant presentation in detail, and only the more striking differences will be mentioned. On cooked rice the pigment of MacMillan's *F. oxysporum* was uniformly of a deeper color, appearing usually as a blue violet to jasper red as compared with light or shell pink with the tobacco-wilt Fusarium. The same was more or less characteristic on oatmeal agar, while on the other media pigment differences were insignificant. A fairly striking difference appeared with respect to the formation of sclerotial masses which came on early and in abundance on potato plugs with the tobacco-wilt Fusarium but only slowly or not at all with the *F. oxysporum* strains on hand. Sporodochia were also produced in abundance with the tobacco-wilt Fusarium on Melilotus stems but did not appear in any of the *F. oxysporum* strains, although they had, no doubt, occurred previously in these strains. In the absence of sporodochia in the cultures of *F. oxysporum* a satisfactory detailed comparison from a morphological standpoint could not, of course, be made. On the basis of certain morphological and cultural differences—that is, pigmentation and sclerotial formation, together with the failure to obtain wilt of the potato, it was at first believed that we were

dealing with a form on tobacco sufficiently distinct from *F. oxysporum* to warrant the creation of a new species. These conclusions were upset, at least for the time being, by the appearance of signs of wilt in one plant of the White Burley tobacco, out of six or eight planted, in soil inoculated with MacMillan's *F. oxysporum*. Several pots of soil were now prepared in December, 1919, and were again infested with several strains of *F. oxysporum* in comparison with my own strains secured from Maryland and Ohio, one of them being a 1916 isolation of the tobacco-wilt Fusarium which had been transferred from an old, dried culture. Good infection (about 80 per cent) was obtained with the tobacco-wilt strains and with MacMillan's strain but not with Bisby's strains (cultures in better growing condition than MacMillan's) nor with Wollenweber's strains (cultures in poorer condition than MacMillan's strain). MacMillan's strain did not, however, prove as virulent as the strains from tobacco, and the symptoms were not identical—that is, the leaves did not uniformly lose their color but presented more of a mottled appearance in the early stages of the disease, and the vascular system was not so distinctly discolored. On plating out the stem and midrib of the infested plants from MacMillan's strain in comparison with the others, the characteristic "sub-normal" condition of MacMillan's strain reappeared, showing that the strains producing the disease were the ones inoculated into the soil. A third series of inoculations was made, using all the strains of *F. oxysporum* at hand. Infection was again obtained with MacMillan's strain and with two of Wollenweber's original strains but not with the others.

In view of these results it appears that strains of *Fusarium oxysporum* may vary considerably as regards pathogenicity, but whether this is a true strain difference or merely one resulting from culturing can not be stated. It was evident, however, that the tobacco-wilt Fusarium had not suffered any loss in virulence from four years in culture, existing for a large part of this time under unfavorable cultural conditions. If *F. oxysporum* is as common in potato fields as a parasite and as common a soil saprophyte as literature would lead us to believe, it is quite surprising to us that wilt of tobacco has not been more generally noted, provided we assume the tobacco-wilt may be caused by *F. oxysporum*, since tobacco and potatoes are frequently grown in close proximity and are frequently rotated. This would be even more surprising when we add that certain varieties of tobacco are apparently more susceptible to the wilt than is the potato.

As has been stated, no infection has been secured on potato with the tobacco-wilt Fusarium, although this may sometime be accomplished. In the early work attempts were also made to get infection on tomato, cowpeas, and cabbage, but without results. Excellent infection has, however, been secured upon *Nicotiana glauca* (California tree-tobacco)

which is very dissimilar to ordinary tobacco. *N. glauca* has, in fact, been the most susceptible plant to tobacco-wilt with which we have worked. Infection has also been secured upon *N. rustica.*

On the basis of this study of the tobacco-wilt Fusarium, it is believed that although *Fusarium oxysporum* from potato is to be regarded as being able to cause a wilt of tobacco, it is not to be regarded as identical with the tobacco-wilt Fusarium as regards pathogenicity. That certain cultural differences exist has also been indicated. The final justification for placing the tobacco-wilt Fusarium as a variety of *F. oxysporum* lies in the small but nevertheless significant morphological differences which have been found to exist. These morphological differences are to be found in the somewhat larger conidia but more particularly in the higher percentages of 4- and 5-septate conidia.

One Fusarium has already been described on tobacco—*Fusarium tabacivorum*, Delac.—and although this species can not be regarded as authentic, it is thought best not to confuse the nomenclature by deriving the variety name from the specific name of tobacco. Furthermore the tobacco-wilt Fusarium is not limited to *Nicotiana tabacum* alone but attacks other members of this genus. It is therefore proposed to derive the third member of the trinomial from the generic name of tobacco. Accordingly the name *Fusarium oxysporum* (Schlecht.) Wr. var. *nicotianae*, n. var. is proposed. The following description is presented.

**Fusarium oxysporum** (Schlecht.) Wr. var. **nicotianae**, n. var.

*Fusarium nicotianae* isolated from wilting tobacco plants (*Nicotiana tabacum L.*) from Maryland and Ohio agrees quite closely morphologically with Wollenweber's diagnosis of *F. oxysporum* (Schlecht.) Wr. except in certain details not readily determined. Mycelium on most media pure white to a light pinkish tinge, of a rather "powdery" appearance, due to presence of numerous microconidia. Blue and light ochraceous salmon-colored sclerotia formed early on steamed potatoes. No true pionnotes observed. Reduced pionnotes or "pseudopionnotes" obtained. Sporodochia produced in abundance on Melilotus stems and on oatmeal agar. These are salmon-colored and when "normal" contain almost entirely 3 to 5-septate conidia, slightly larger than those of *F. oxysporum*. Three-septate conidia up to 100 per cent 34.9 by 4.2 microns (25.0 by 3.7 microns to 45.4 by 4.6 microns). Four-septate up to 40 per cent, 39.3 by 4.0 microns (29.6 by 3.7 microns to 46.3 by 4.6 microns). Five-septate up to 18 per cent 44.3 by 4.0 microns (38.9 by 3.7 microns to 51.1 by 4.1 microns). Six-septate very rare. Non-septate conidia in old sporodochia rare (7.1 by 2.4 microns). One-septate equally rare (10.1 by 2.7 microns). Two-septate up to 4 per cent (18.5 by 3.7 microns). Non-septate spores from mycelium 8.1 by 3.4 microns (10.2 by 3.7 microns to 3.7 by 2.7 microns). Chlamydospores terminal, intercalary and conidial, smooth, round, frequently in masses 8.2 microns (6 to 10.2 microns).

Pigment production not so deep as in most descriptions of *Fusarium oxysporum.*

HABITAT.—Parasitic in fibro-vascular bundles of *Nicotiana tabacum* in Maryland and Ohio, causing a decided wilting of plants followed by death. Also produces a similar disease of *N. glauca* and *N. rustica* by artificial inoculation.

## CONDITIONS INFLUENCING THE DISEASE

A thorough study of the environmental conditions influencing the occurrence and extent of injury by the Fusarium-wilt disease has not been undertaken. Some evidence has been obtained, however, through experimental work and observation which is of interest in this connection. The progress of experimental work along this line was interfered with by the difficulty of obtaining a high percentage of infection even under favorable conditions, so that a considerable number of plants would have to be grown to obtain good data. This very fact should in itself stimulate further research along this line, since it is evidence that the environmental conditions most conducive to parasitism are not fully understood. Where a number of factors are involved, however, this subject becomes exceedingly complex. The introduction of such a factor as wounding, which may occur "naturally" or may vary in considerable degree when produced artificially, is a complicating factor in the tobacco-wilt disease, which in some respects renders it unfavorable for such a study.

The evidence for the conclusions presented in this paper will not be given in detail. The methods of investigation were essentially the same as those which were used in a study of the influence of soil environment on the rootrot of tobacco as described by Johnson and Hartman (5). The soil used was, however, artificially infested from pure cultures following steam sterilization. The object of the soil sterilization has been partly to secure better infestation of the soil following inoculation. In practically all cases the inoculum has been grown on 100 parts sand, 10 parts of corn meal, and 1 part of glucose to 50 parts of water. A heavy growth of mycelium and an abundance of spores on this medium undoubtedly suffice to inoculate the soil thoroughly, as is shown by instances in which "100 per cent infection" occurs (Pl. 63, B. C). Where a uniform infestation of the soil is not required, rapid infection can be secured by using conidia and mycelium from ordinary cultures placed in the soil about the wounded stems.

As will be shown later, the White Burley variety of tobacco was found to be the most susceptible to the Fusarium-wilt disease; therefore, this variety was used in all cases in the environmental studies. The use of other more resistant varieties would have rendered the securing of results far more difficult. It is assumed, however, that the same relative results would have been secured with the more resistant varieties.

The seedlings were in all cases transplanted into the infested soil from steam-sterilized soil. The root systems especially were therefore in all cases more or less wounded in their removal from the soil. Although infection has been observed in seedlings not transplanted, it is quite certain that the tobacco-wilt organism is largely dependent upon wounded

host tissue for initial infection. However this may be, it was found that wounding the plant greatly increased the possibilities of infection, and in some of the later experiments the plants were all wounded, usually by pinching or pulling off two basal leaves, in addition to the "natural" wounding resulting from transplanting.

SOIL TEMPERATURE.—Four series of experiments were run in the soil temperature control "tanks" during the winters of 1918–19 and 1919–20 in a manner similar to that which has been described for the Thielavia rootrot studies (5). Two plants in uninfested soil and two in infested soil were grown at each temperature. The temperatures usually ranged from 15° to 38° C., with intervals of 2°—that is, 12 different temperatures were used. The results in two of the trials were not convincing on account of a low percentage of infection, although the later results were approximated. In one series wilt occurred only at the approximate temperatures of 28° and 32° and not at the intermediate temperature used. In the other case, wilt occurred only at 26° to 27° and 30° to 32°. These results can only be said to indicate roughly that the higher soil temperatures are more favorable than the lower temperatures.

In the third experiment, however, more uniform infection was secured. Signs of disease were first evident at 28° to 29° and 25° to 26° C., and these were soon followed by disease at 26° to 27°, 30° to 31°, 23° to 24°, and 21° to 22°. Eighteen days later all plants in the infested soil were dead at all temperatures between 21° to 22° and 30° to 31° and also at 32° to 33°. One plant was dead and one diseased at 31° to 32°, 34° to 35°, 19° to 20°, and 17° to 18°, and two were slightly diseased at 15° to 16°. The most favorable temperature for infection and progress of the wilt appeared to be between 25° and 30°, but it seemed evident that a wide range of temperature existed within which the disease could occur.

The surface soil of the pots in the first three experiments was not insulated, though it should be, particularly in diseases of this sort where the parasite is systemic; therefore, it is quite likely that infection may have occurred near the surface where for short periods the temperature varied considerably from those given, particularly at temperatures above 30° C. Difficulties are encountered in controlling soil temperatures sufficiently accurately at all points in the soil containers in dealing with a systemic disease, though these difficulties do not play so large a rôle in cases where a parasite is limited entirely to subterranean parts. In a fourth test, using soil temperatures 3° apart, in which special attempts were made to keep the temperature at the surface of the soil constant by means of glass covers and shading of the jars in the tanks, wilt occurred first and most abundantly at 30° to 31°, and no wilting occurred at 13° to 14° or at 35° to 36°. We feel confident in concluding from the results of these experiments that the optimum temperature for the disease lies between 28° and 31°—that is, the Fusarium-wilt organism is

a warm-weather parasite, and at lower temperatures the likelihood of its occurrence is diminished. (Pl. 67, I and II.)

It is significant that the optimum for the growth of the wilt Fusarium in culture was also found to be between 28° to 30° C. Growth was very slow at 10°, and no growth was obtained at 7° and 35°. Since no growth occurred in culture at 35°, it is seemingly quite evident that no infection should occur above 35° and that chances of infection probably are considerably reduced before that temperature is reached.

It may now be recalled that the Fusarium-wilt of tobacco was first brought to our attention in 1916, when it apparently was assuming serious proportions, although it had been previously noted by the growers in lesser amounts. It will also be remembered that the summer of 1916 was one of the warmest seasons recorded by the Weather Bureau stations throughout the country, and that the soil temperature was correspondingly high that season, as shown, for instance, by records taken at depths of 2, 4, and 8 inches at Wisconsin (5). The season of 1919, when the disease occurred in Ohio, was also relatively warm. Though the evidence is scanty for the occurrence of the disease under field conditions, there is no doubt a correlation with high soil temperatures.

SOIL REACTION.—In experiments with Thielavia a series of soil cultures was prepared and described (5) in which the reaction of a soil of very high acidity was changed by adding varying amounts of calcium carbonate so as to give different reactions ranging from high acidity (9.38 tons lime required per acre) to one of high alkalinity. These soils have changed somewhat in reaction during the two years in which they have been used, but, as shown by the Troug color test, the same relative reaction was probably maintained. The determination of the reaction of these soils by the hydrogen-ion method, however, indicated that high alkalinity was apparently not obtained, the $P_H$ value ranging from 5.4 to 7.2. These soils were sterilized, and one series in duplicate was inoculated with *Fusarium oxysporum* var. *nicotianae*, the other series being left as uninfested controls. Tobacco seedlings of the White Burley variety were then transplanted into them. Three separate trials were run, two of which gave reliable results, and one yielded unreliable results because of poor infection. In one experiment all the plants died at the three highest soil acidities, one died in each of the next three lower reactions, and none died in the three jars at the alkaline end, although finally they all became infected. In another experiment all plants died in the first five grades of reaction from the acid end and one in each of series 6, 8, and 9, but none in the seventh, although they were both infected. The evidence seems fairly conclusive that an acid soil favors the wilt disease, although it may occur in neutral or alkaline soils. The crocks were, however, watered from the top, and part of the soluble salts were washed downward. The soil may not have been of the same reaction throughout for this reason, but the difference could

not have been great, since the soils were repeatedly mixed and stirred. Infection apparently occurred within a wide range of soil reaction, although it was strikingly more pronounced at the higher acidities (Pl. 67, III). For this reason we can not agree with MacMillan (*8*) that infection with Fusarium is favored by alkaline soils. The behavior of Fusarium in the experiments described is also in line with the results secured in the culture of *F. oxysporum* var. *nicotianae* in culture media of varying reaction.

The Fusarium-wilt organism was inoculated in tubes of beef broth at reactions ranging from −5 per cent to +5 per cent. After 5 days the best growth was at +1. After 12 days it was apparently growing best at +3 and poorest at +5, but after about 40 days the fungus growth seemed most profuse at +5. On potato agar, however, the best growth was obtained at neutral to +0.7. After 8 days there was decidedly poorer growth as alkalinity was increased as well as a retarded growth at +1 per cent. This fungus, in common with most forms, is not favored by alkaline media, and there seems to be no good reason for expecting it to be more virulent in alkaline soils.

OTHER ENVIRONMENTAL CONDITIONS.—With respect to other environmental conditions, we are able to say very little. Observation seems to indicate that high soil moisture is not especially favorable to the disease. Infection has been noted incidentally in both relatively dry and moist soils, but the writer has been of the opinion that the soil should be kept relatively dry to get good artificial infection. The disease in Maryland occurred on high, sandy land, and the two years, 1916 and 1919, in which the disease was called to the writer's attention were both both notably hot and dry.

A single trial with soils ranging from no organic matter to pure leaf mold did not indicate any decided preference on the part of the disease for the presence of organic matter in the soil.

In the soil-inoculation experiments it has appeared that the highest infection has always been secured by planting to tobacco soon after the inoculation of the soil. Later plantings in the same soil usually resulted in a lower percentage of infection. The parasite apparently does not find the soil a very favorable medium for maintaining itself, even in the presence of host plants, and in their entire absence it probably gradually dies out completely. Nothing definite is known, however, as to how long the fungus may persist in the soil.

To summarize briefly, the conditions which seem most necessary for good infection and progress of the disease are:

1. Heavy soil infestation.
2. Wounded host tissue, particularly of stems below the surface of the soil.
3. A relatively high temperature.
4. A susceptible variety.

## VARIETAL RESISTANCE TO WILT

The early infection experiments indicated that a difference in varietal resistance to the Fusarium-wilt probably existed in tobacco, but facilities for carrying out varietal tests under field conditions were not readily obtainable. It was therefore decided that preliminary tests would be carried out on a small scale, using artificially inoculated soil in greenhouse "flats" (boxes 16 inches by 24 inches and 3 inches deep). Twelve to 14 of these flats were filled with greenhouse soil and sterilized at 100° C. for two hours. When cooled, each flat was inoculated by mixing into it a sand-cornmeal culture previously referred to, after which the soil in all the flats was dumped together and again thoroughly mixed to obtain uniform infestation, and the flats were again filled. Twenty plants of each variety used were then transplanted into each flat from the sterilized soil in which they had been grown. Three series of tests were carried out, two out of doors in the summers of 1918 and 1919 and one in the greenhouse in November, 1918. In the first two tests no special attempt at artificial wounding was made. In the last series the plants were wounded by pinching off two basal leaves from each plant. Relatively higher infection was obtained in this manner. The varieties tested represent practically all the types grown commercially in the United States, and a few others, including two other species, *Nicotiana glauca* and *N. rustica*, and in one instance also an $F_1$ of a cross between a White Burley resistant to Thielavia rootrot and Fusarium-wilt and one susceptible to these diseases. The experiments were terminated about one month after transplanting. In taking notes on the results it was found convenient to grade the individual plants into one of four classes: 1, dead; 2, badly diseased; 3, slightly diseased; 4, healthy.

If a plant was completely wilted and dried it was classed as dead. All remaining plants showing any exterior symptoms of disease were classed as badly diseased. The remainder of the plants were then cut off close to the root system, slit longitudinally, and examined for discolored vascular systems. If any discoloration occurred attributable to infection, the plant was listed as slightly diseased, and if none occurred it was placed in the healthy class. In this manner the classification included the important conditions and yet was not wholly arbitrary. The results of the three tests are shown in Table I. In order to average these data and to arrive at a fair average figure for relative resistance expressed on the percentage basis, a more or less arbitrary formula was established. This method may be briefly described as follows: If a plant remained healthy it was credited with three points; if slightly diseased, 2 points; if badly diseased, 1 point; and if dead it was rated at zero. Twenty seedlings in one flat all healthy would be credited with 60 points ($20 \times 3$), which is the maximum given and corresponds to 100 per cent resistance. Twenty seedlings in one flat all dead would receive

no credit (20×0), which is the minimum and equals 0 per cent resistance, or 100 per cent susceptibility. On the other hand, if, out of 20 plants in a flat, 5 were dead, 5 badly diseased, 5 slightly diseased, and 5 healthy, 30 points would result (5×0=0, 5×1=5, 5×2=10, 5×3=15, total 30) which is 50 per cent resistance.

It is only in some such manner, in fact, that resistance could be fairly recorded in figures. Comparative yield of plants would give no better criterion, since a plant might be infected and show no depreciation of yield and might even reach maturity and be badly diseased without appreciably influencing yield.

The average resistance given is on the basis of only 60 plants, except in a few instances when it is on a basis of only 40 or 20 plants. Though the numbers are small, they are believed to be more significant than could be obtained under field conditions with a greatly increased number of plants, because of the uniformity of the soil and of infestation.

From these calculations it will be noted that none of the varieties tried were absolutely immune. The most resistant varieties are the Connecticut Havana, Cuban, and Sumatra, with 98 per cent resistance. Since the figures are not regarded as significant within about 5 per cent, the Pennsylvania Broadleaf and the Wisconsin binder selection H12074, a strain selected for resistance to rootrot due to *Thielavia basicola*, should be included in this group. The least resistant of the *Nicotiana tabacum* varieties is the ordinary White Burley (32 per cent) (Pl. 67, IV). Strangely enough, *N. glauca*, perhaps the species farthest removed from *N. tabacum* in similarity, is the least resistant (23 per cent) to *F. oxysporum* var. *nicotianae* of all plants tried. The varieties listed have been repeatedly tried out for their resistance to the rootrot of tobacco due to *T. basicola* (3), and it is interesting to note the correlation in resistance to the two parasites. *N. rustica* is immune to Thielavia but may be attacked by Fusarium. Shade-grown Cuban, Little Dutch, and Wisconsin selection H12074 are very resistant to Thielavia, but, while Little Dutch is not very resistant to Fusarium, the other two are decidedly resistant. The Pryor and Oronoco types are very susceptible to Thielavia but relatively resistant to Fusarium. The White Burley, which is most susceptible to Thielavia, is also most susceptible to Fusarium. A strain of White Burley selected for resistance to Thielavia is also fairly resistant to Fusarium. The $F_1$ generation of a cross between resistant and susceptible Burley is seemingly intermediate in resistance to Fusarium-wilt, as it is to Thielavia. The figures for the latter are, however, not large enough to be of much significance. The cases cited seem to be sufficient to warrant the statement that the correlation between resistance in tobacco to *Thielavia basicola* and to *F. oxysporum* var. *nicotianae* is low.

TABLE 1.—*Relative resistance of varieties and strains of tobacco to Fusarium-wilt*

| Variety or strain. | Experiment I. | | | | | Experiment II. | | | | | Experiment III. | | | | | Average relative resistance. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dead. | Badly diseased. | Slightly diseased. | No disease. | Relative resistance. | Dead. | Badly diseased. | Slightly diseased. | No disease. | Relative resistance. | Dead. | Badly diseased. | Slightly diseased. | No disease. | Relative resistance. | |
| Connecticut Havana | 0 | 0 | 2 | 18 | 97 | 0 | 0 | 0 | 20 | 100 | 0 | 0 | 1 | 19 | 98 | 98 |
| Texas Sumatra | | | | | | | | | | | 0 | 0 | 1 | 19 | 98 | 98 |
| Shade-grown Cuban | 0 | 0 | 0 | 20 | 100 | 0 | 1 | 1 | 18 | 95 | 0 | 0 | 0 | 20 | 100 | 98 |
| Wisconsin selection H12074 | 0 | 0 | 2 | 18 | 97 | 0 | 0 | 0 | 20 | 100 | 0 | 2 | 2 | 16 | 90 | 96 |
| Pennsylvania Broadleaf | 0 | 1 | 0 | 19 | 97 | 0 | 1 | 0 | 19 | 97 | 1 | 1 | 2 | 16 | 88 | 94 |
| Narrowleaf Oronoco | 0 | 1 | 1 | 18 | 95 | 0 | 2 | 1 | 17 | 92 | 0 | 2 | 2 | 16 | 90 | 92 |
| Blue Pryor | 0 | 1 | 3 | 16 | 92 | 0 | 4 | 0 | 16 | 87 | 2 | 3 | 1 | 14 | 78 | 86 |
| Connecticut Broadleaf | 0 | 1 | 3 | 16 | 92 | 0 | 0 | 0 | 20 | 100 | 2 | 2 | 4 | 12 | 77 | 90 |
| Little Dutch | 0 | 3 | 5 | 12 | 82 | 1 | 1 | 2 | 16 | 88 | 5 | 7 | 2 | 6 | 48 | 73 |
| Maryland Broadleaf | 4 | 3 | 9 | 4 | 55 | 0 | 2 | 3 | 15 | 88 | 8 | 6 | 1 | 5 | 38 | 80 |
| White Burley | 9 | 3 | 4 | 4 | 38 | 9 | 2 | 4 | 5 | 41 | 12 | 5 | 3 | 0 | 18 | 32 |
| Resistant White Burley[a] | 1 | 2 | 10 | 7 | 71 | 0 | 2 | 3 | 15 | 88 | | | | | | 80 |
| $F_1$ (Resistant[a] × susceptible White Burley) | | | | | | 1 | 8 | 6 | 5 | 58 | | | | | | 58 |
| *Nicotiana rustica* | | | | | | 2 | 1 | 2 | 15 | 83 | 0 | 0 | 0 | 20 | 100 | 92 |
| *Nicotiana glauca* | | | | | | 3 | 13 | 0 | 4 | 41 | [b] 19 | 0 | 0 | 1 | 5 | 23 |

[a] Resistant to Thielavia rootrot.

[b] Not certain all died from wilt.

No work has been done upon the selection of resistant strains within individual varieties with the object of controlling the Fusarium-wilt disease of tobacco. From the data presented, however, it is obvious that this is a logical procedure in the control of this disease, should its economic importance warrant the undertaking. The evidence at hand indicates that the White Burley which was selected for its resistance to Thielavia rootrot also shows marked resistance to Fusarium-wilt, as compared with the ordinary White Burley, although the selection was made, of course, without reference to resistance toward Fusarium. This is, in fact, a step in the direction of control should the Fusarium-wilt become serious in the White Burley section, where, because of the susceptibility of the ordinary strains grown, it is most likely to become of economic importance. Selections in the Maryland Broadleaf variety, which is the next most susceptible of the commercial types, seems entirely feasible. Since it is on this type grown in Maryland that the disease has apparently been most common, it may be advisable in the near future to undertake to select a resistant strain of this variety unless other control measures are found which are more readily applicable.

## CONTROL MEASURES

In the absence of the use of resistant varieties or strains, there appear to be only the ordinary measures of control applicable to plant parasites infesting the soil. Since the disease is due to a living organism which is carried over in the soil from year to year either as a parasite on the tobacco plant or existing as a saprophyte in the vegetable matter of the soil for possibly a limited number of years, the most evident measure of control seems to be the avoidance of infested soil. Especially when planting on new ground free from disease, it is advisable to be certain also that the seedlings to be used have not been grown on infested soil, since the parasite may be transmitted to the new soil in this manner. Using new ground for seed beds or thoroughly sterilizing old ground by means of steam is therefore desirable. New fields or seed beds receiving surface drainage water from old, infested fields should also be avoided, as should any unnecessary farm operation capable of carrying even relatively small amounts of soil from infested fields to uninfested ones. Where relatively few plants in a field are infected and show the disease, it is a good precaution to remove these plants together with the roots and to burn them so as to decrease the amount of infestation.

## SUMMARY

(1) A disease of tobacco, apparently previously undescribed, has been found to occur in Maryland and Ohio. The disease is characterized by a yellowing and wilting of the leaves of the plant, usually followed by death of the entire plant. The fibro-vascular system of infected plants is characteristically brown or black.

(2) A species of Fusarium can be readily isolated from the discolored area, and infection of seedlings can be produced by inoculating the soil with this fungus. The causal organism is shown by stained paraffin sections to exist throughout the fibro-vascular system of infected parts.

(3) The Fusarium concerned seems to be closely related to *Fusarium oxysporum* (Schlecht.) Wr. but differs somewhat from this species in morphology, physiology, and pathogenicity.

(4) Infection has been secured with two strains of *Fusarium oxysporum* from potato on tobacco but has not been secured with the tobacco strain on potato.

(5) The trinomial *Fusarium oxysporum* (Schlecht.) Wr. var. *nicotianae*, n. var., is proposed for the tobacco-wilt organism.

(6) The conditions favoring infection with the tobacco-wilt organism are heavy soil infestation, wounded host tissue, a relatively high soil temperature (28° to 31° C.), and a susceptible variety.

(7) It has been found that varieties of tobacco differ markedly in their resistance to Fusarium-wilt. The White Burley variety is most susceptible, and the Havana Seed and Cuban varieties are among the most resistant.

(8) Where the disease threatens to become serious, growers are advised not to grow tobacco on the infested soils and to avoid the danger of infested seed beds. The most hopeful means of control appears to lie in the development of strains resistant to the disease within the various susceptible varieties.

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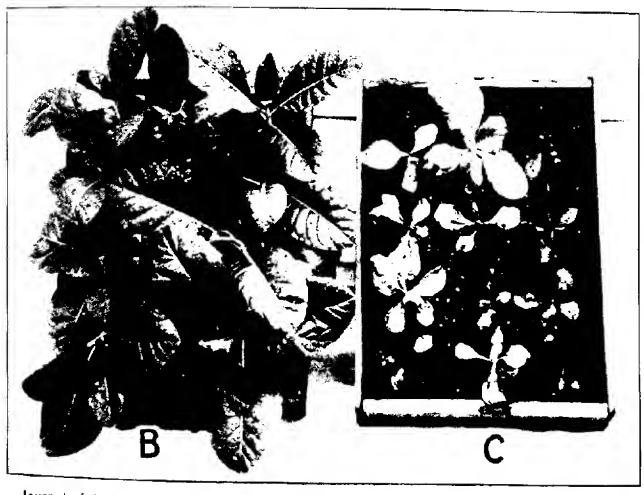
PLATE 63

A.—A typical spot in a field of Maryland Broadleaf tobacco infested with Fusarium wilt. Benedict, Md. 1916.

B.—Uninoculated control.

C.—Plants grown in soil artificially inoculated with the tobacco-wilt Fusarium and planted to White Burley.


A


B
C


B


A

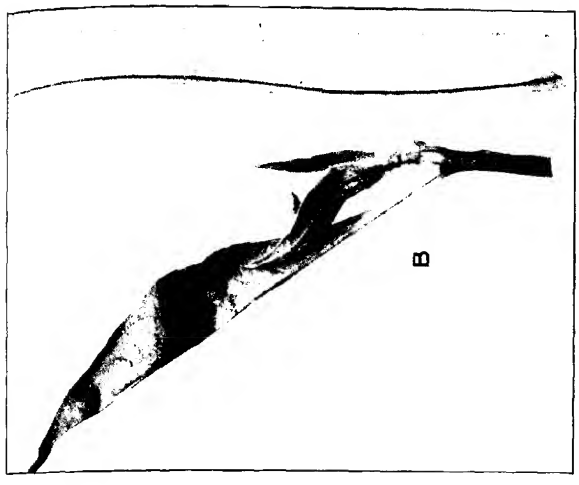
PLATE 64

A.—Plant infected with Fusarium-wilt, showing wilting in vertical line on stalk.
B.—Last stages of Fusarium-wilt in Maryland Broadleaf tobacco.

PLATE 65

A.—Result of plating out five pieces of infected vascular tissue from infected plant, illustrating character of growth of mycelium on potato agar.

B.—Stem and midrib of plant, cut longitudinally to show the blackened vascular system.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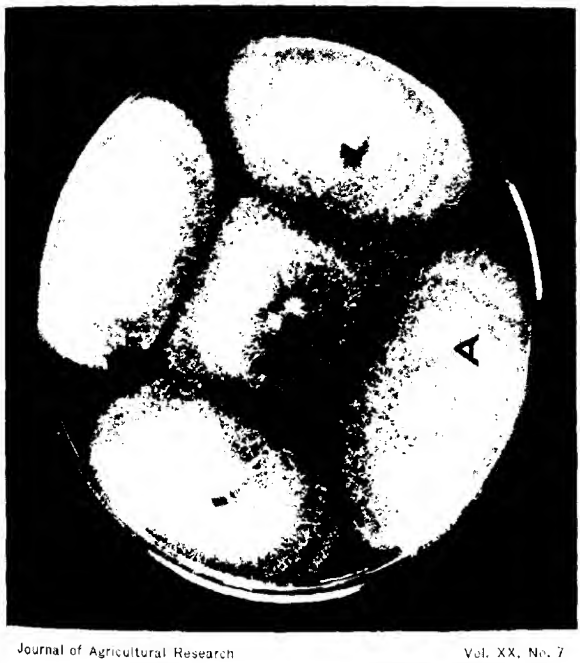
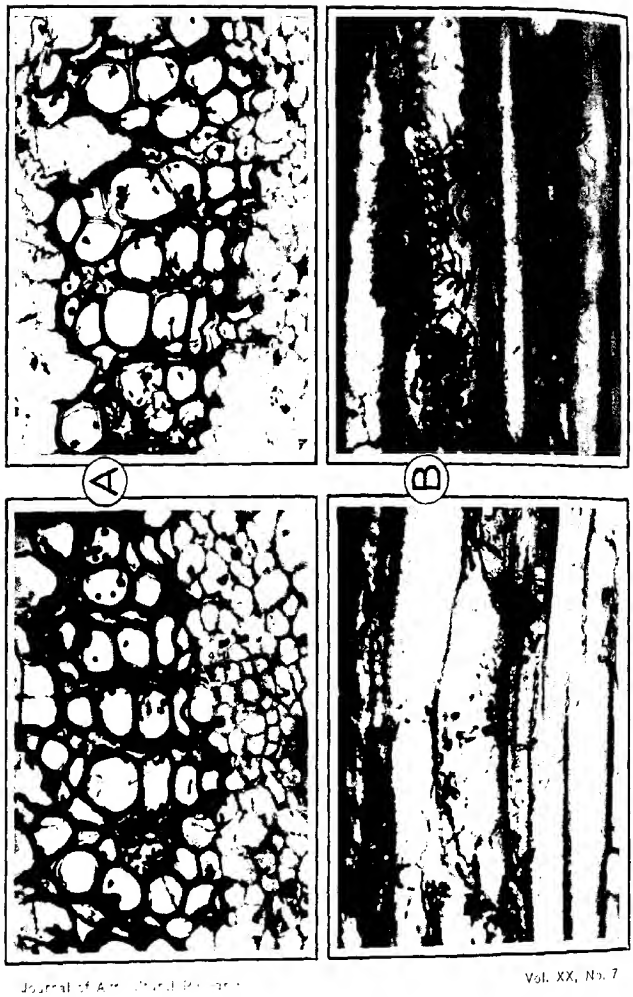

A


A
B

## PLATE 66

A.—Cross sections through vascular system of tobacco plant infected with Fusarium-wilt, showing the fungus mycelium in the vessels. Pianese stain.

B.—Longitudinal sections through the vascular system of plants infected with Fusarium-wilt, showing the fungus strands in the vessels. Pianese stain.

## PLATE 67

I.—Plants illustrating the influence of soil temperature on degree of wilting of plants in soil infested with Fusarium-wilt. The plants were grown at the following soil temperatures:

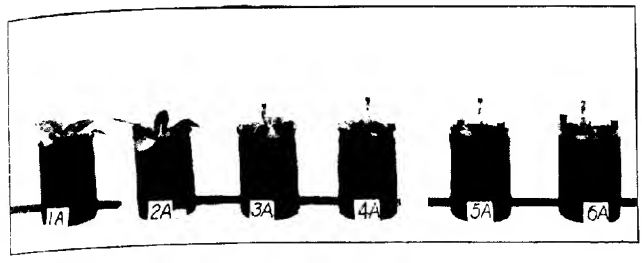
1A, 15° to 17° C.
2A, 19° to 20° C.
3A, 22° to 24° C.
4A, 26° to 28° C.
5A, 29° to 31° C.
6A, 32° to 34° C.

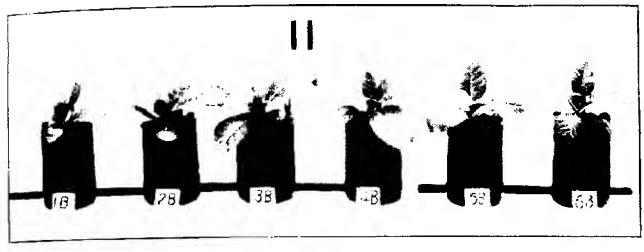
The upper limit for infection is close to 35°. Infection has occurred at 19° to 20°, but the progress of the disease is very slow.

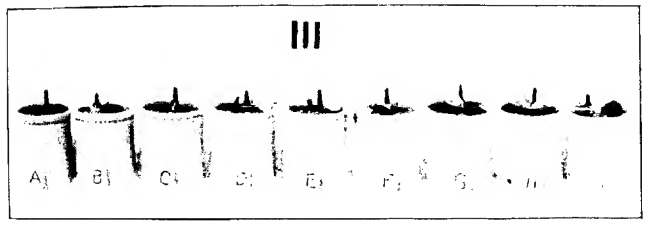
II.—Plants grown in the same soil uninfested and at corresponding soil temperatures.

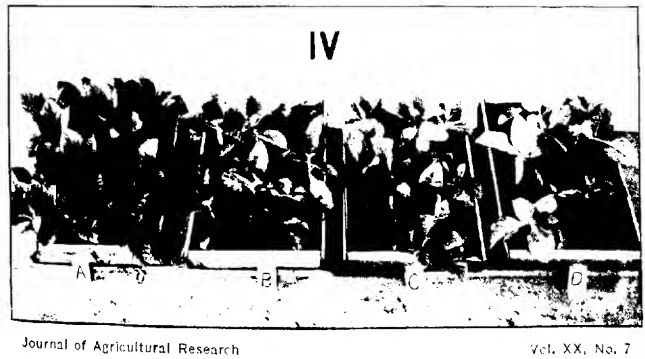
III.—Plants illustrating the influence of varying soil reaction on the amount of Fusarium-wilt in infested soil. A, highest acidity (medium to strong) to E near neutral and I alkaline end. Same soil (selected for high acidity) in all crocks but brought to various reactions by addition of precipitated calcium carbonate.

IV.—Plants illustrating varietal differences in resistance of tobacco to Fusarium-wilt. Soil artificially inoculated, uniformly mixed, and transplanted with 20 plants each of the following varieties: A, Connecticut Havana; B, Little Dutch; C, Maryland Broadleaf; D, White Burley.


1A
2A
3A
4A
5A
6A


II
1B
2B
3B
4B
5B
6B


III


IV
A
B
C
D

# SUGAR BEET TOP SILAGE

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The growing of sugar beets in the Pacific Northwest for the manufacture of sugar is rapidly becoming a major occupation, but the beet root from which the sugar is produced is not the only source of revenue when sugar beets are grown. There remains for the farmer a considerable portion of the crop in the form of sugar beet tops, which represent a large amount of value as a feed for stock. In recent years the farmer has utilized this source of feed, thereby securing additional revenue in the form of live stock and also in increased fertility of the soil.

It is estimated that a normal crop of sugar beets produces from 50 to 60 per cent of the weight of the crop in the form of salable beets and the remaining percentage in beet tops. This being true, it is evident that beet tops furnish no mean supply of feeding stuff for the farmer, and the careful preservation of this by-product of the beet-growing industry should be practiced.

The older countries many years ago realized the food value contained in the by-products of the sugar beet industry. Many methods have been used for the preservation of the sugar beet tops, but the siloing has received the popular choice because more food value is retained by this method than by any other. In the United States, siloing sugar beet tops has been practiced for many years. Recently the United States Department of Agriculture [2] has estmated that beet tops, when properly siloed and when fed with alfalfa hay, will reduce the hay requirement by approximately one-half. With the high prices of hay that have prevailed for the past few years, it is evident that the proper preservation of beet tops is a subject of no little economic importance.

During the past two years, numerous instances have come to the writer's notice of stock dying when fed beet top silage, and the cause of their death was attributed to the feeding of this product. However, since thousands of head of stock are successfully fed on this silage, it appeared to the writer that the fatalities were due mainly to the feeding of abnormal rather than normal silage. With the idea in mind of securing knowledge of the chemical nature of the average beet top silage as found on the average farm in the sugar beet districts, several samples of

[1] Published by the permission of Director E. J. Iddings, Idaho Agricultural Experiment Station.

[2] Jones, James W. BEET-TOP SILAGE AND OTHER BY-PRODUCTS OF THE SUGAR BEET. U. S. Dept. Agr. Farmers' Bul. 1095, 24 p., 12 fig. 1919.

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silage were collected and sent in to the chemistry department of the Idaho Agricultural Experiment Station.[1]

In the fall of 1918, four samples of beet top silage were collected from the southern part of the State by Mr. Rinehart. In 1919, six samples were collected by Mr. Aicher. All samples are representative of the average silage made in Idaho. An approximate analysis was made on each of these samples. In addition volatile and nonvolatile acid determinations were made on several of these samples of silage. The results of the approximate analysis are given in Tables I and II. Table I gives the results on the wet basis—that is, on the basis of the original moisture content—and Table II the results on the anhydrous or moisture-free basis.

TABLE I.—*Analysis of 100 gm. sugar beet top silage containing moisture*

| Sample No. | Moisture. | Dry material. | Total residue left on ignition (dirt and ash). | Dirt. | Ash. | Protein. | Ether extract. | Crude fiber. | Carbohydrates (by difference). | Quality of silage. |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Per ct.* | *Per ct.* | *Per cent.* | *Per ct.* | *P. ct.* | *Per ct.* | *Per ct.* | *Per ct.* | *Per cent.* | |
| 1 | 82.5 | 18.5 | 7.04 | 5.09 | 1.95 | 2.18 | 0.48 | 1.52 | 7.28 | Poor. |
| 2 | 76 | 24 | 5.29 | 2.32 | 2.97 | 2.95 | .85 | 3.05 | 11.86 | Fair. |
| 3 | 59 | 41 | 17.27 | 12.79 | 4.48 | 4.40 | .92 | 3.44 | 14.97 | Poor. |
| 4 | 80 | 20 | 10.51 | 8.42 | 2.09 | 1.88 | .48 | 1.43 | 5.70 | Do. |
| 5 | 49.5 | 50.5 | 25.65 | 18.39 | 7.26 | 6.51 | .68 | 3.89 | 13.77 | Do. |
| 6 | 68.5 | 31.5 | 11.80 | 7.09 | 4.71 | 4.29 | 1.14 | 2.70 | 11.57 | Fair. |
| 7 | 70 | 30 | 19.08 | 14.46 | 4.62 | 2.04 | .53 | 1.91 | 6.44 | Poor. |
| 8 | 70 | 30 | 9.79 | 5.13 | 4.66 | 4.38 | .84 | 2.60 | 12.39 | Fair. |
| 9 | 78.2 | 21.8 | 14.13 | 11.65 | 2.48 | 1.38 | .26 | 1.10 | 4.93 | Poor. |
| 10 | 74.4 | 25.6 | 12.22 | 8.26 | 3.96 | 2.71 | .29 | 2.00 | 8.38 | Do. |

TABLE II.—*Analysis of 100 gm. moisture-free sugar beet top silage*

| Sample No. | Total residue left on ignition (dirt and ash). | Dirt. | Ash. | Protein. | Ether extract. | Crude fiber. | Carbohydrates (by difference). |
|---|---|---|---|---|---|---|---|
| | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| 1 | 38.04 | 27.51 | 10.53 | 11.80 | 2.57 | 8.22 | 39.37 |
| 2 | 22.04 | 9.65 | 12.39 | 12.28 | 3.54 | 12.69 | 49.45 |
| 3 | 42.12 | 31.20 | 10.90 | 10.74 | 2.24 | 8.38 | 36.51 |
| 4 | 52.55 | 42.10 | 10.45 | 9.40 | 2.40 | 7.15 | 28.50 |
| 5 | 50.81 | 36.41 | 14.37 | 12.89 | 1.35 | 7.70 | 27.26 |
| 6 | 37.45 | 22.50 | 14.95 | 13.61 | 3.62 | 8.57 | 36.75 |
| 7 | 63.59 | 48.19 | 15.38 | 6.79 | 1.76 | 6.36 | 21.50 |
| 8 | 32.60 | 17.08 | 15.52 | 14.59 | 2.80 | 8.66 | 41.35 |
| 9 | 64.82 | 53.44 | 11.38 | 6.35 | 1.17 | 5.17 | 22.19 |
| 10 | 47.73 | 32.26 | 15.47 | 10.58 | 1.12 | 7.81 | 32.70 |

An examination of the results shows that only three of the samples were classed as fair silage. The remaining seven samples were classed as beet top silage of poor quality. A noteworthy fact seen from the inspec-

[1] The collecting of the samples was made possible through the kind cooperation of Mr. E. F. Rinehart, Field Animal Husbandryman for Idaho, and Superintendant L. C. Aicher, of the Aberdeen substation. The writer wishes to thank these men for their careful notations of general conditions and their interest and cooperation in the work.

tion is the high percentage of dirt or sand found in the residue after ashing. The real or true ash of the beet top silage was separated from the total residue after igniting in an electric furnace, the difference representing sand or dirt. Even on the basis of the silage containing the original moisture it is seen that the percentage of dirt is high in the three samples classed as fair silage, the amount ranging from 2.32 pounds to 7.09 pounds on the basis of the wet silage. When calculated on the moisture-free basis these samples contain dirt and sand to the amounts of 9.65 and 17.1 pounds per 100 pounds of moisture-free silage. On the other hand, the amount of dirt found in the poorer grades of silage ranges on the wet basis from 8.26 pounds to 18.39 pounds per 100 pounds of wet silage, while on the basis of 100 pounds moisture-free silage there are found from 22.50 to 53.44 pounds. These figures are all the more striking when applied to the average daily amount of beet top silage eaten by stock. An animal consuming an average ration containing 35 pounds of beet top silage must necessarily consume from 2.89 pounds to 6.44 pounds of dirt. It is not unfair to assume that such quantities of dirt, which in most localities engaged in growing sugar beets is a light, sandy, volcanic ash, would tend to produce serious digestive disturbances which in turn might produce the death of the animal. In samples 4 and 9, death of stock did actually take place while the silage was being fed. An inspection of the dirt content of these two silages shows a dirt content of 8.42 and 11.65 pounds in every 100 pounds of wet silage and 42.1 and 53.44 pounds, respectively, in every 100 pounds of moisture-free silage.

The reasons for the presence of such a large quantity of dirt in the silage are many. A brief summary of the methods used by the average farmer when siloing sugar beet tops will be given, since it will tend to explain the large quantities of sand and dirt that are present. In the first place, the type of silo is very crude. Usually it is a shallow dirt trench or pit of sufficient size to accommodate the crop of beet tops. The beet tops are thrown into piles in the field and scooped upon wagons. More or less dirt clings to the beet tops, especially if this work is carried on in rainy weather. The wagons are driven into the trench and dumped, each load tending to pack the beet tops previously unloaded. Such procedure does not hinder but rather aids in the carrying in of some dirt. It is readily seen that the whole process of siloing sugar beet tops is one where dirt is collected in all steps of the process from the time of topping the beets until the tops are actually siloed, unless extreme care is used to keep out excess dirt. Without extreme care a good silage can not be obtained. The United States Department of Agriculture has recently issued a bulletin [1] which sets forth the best methods of siloing sugar beet tops and describes the best types of pit silos. Pit silos with concrete side are recommended. Many good suggestions as to the proper

[1] JONES, James W. OP. CIT.

methods that a farmer should use are given. From the study of Tables I and II of this paper it is plain that more care is needed on the part of the average farmer before he can expect to secure a silage of good quality. If the suggestions embodied in farmer's bulletins of the United States Department of Agriculture are followed, the farmer will not only be rewarded with a silage of good quality and high feeding value but he will also avoid the loss of stock.

## ACIDITY OF SUGAR BEET TOP SILAGE

Investigations of many types of silage by the writer [1] and others have indicated that in practically all silages that have undergone a normal fermentation there results an acidity in which the chief acids are lactic, acetic, and propionic, their relative importance decreasing in the order named. In the sugar beet top silage it was desired to study the acidity of several samples to learn what types of acids were formed in the silage found on the average farm. With this idea in mind, several of the samples sent in to the experiment station were examined. The Duclaux method [2] was used for estimating the volatile acids, and the zinc lactate method was used for the nonvolatile or lactic acid. The algebraic and graphic methods described by Gillespie and Walters [3] were used in calculating the individual volatile acid after they were identified by the qualitative tests suggested by Dyer.[4] The results on the volatile and nonvolatile acids are given in Tables III and IV. Table III gives the results on the wet basis and Table IV gives the results on the moisture-free basis.

An inspection of Tables III and IV shows that the acids developed in the sample of sugar beet top silage are not similar to those usually found in the corn silage. Corn silage contains lactic, acetic, and propionic acids. The proportion of lactic to the two volatile acids is usually about 1 part to 75 hundredths, while the proportion of acetic to propionic is usually 1 part to one-tenth. Butyric acid was never found in silage that was classed as good corn silage. It was found, however, in partially spoiled samples. Hence the conclusion was reached that silage containing butyric acid has undergone an abnormal fermentation.

[1] NEIDIG, RAY E. ACIDITY OF SILAGE MADE FROM VARIOUS CROPS. *In* Jour. Agr. Research, v. 14, no. 10, p. 395-409. 1918. Literature cited, p. 408-409.

[2] DUCLAUX, E. RECHERCHES SUR LES VINS. DEUXIÈME MÉMOIRE: SUR LES ACIDES VOLATILS DU VIN. *In* Ann. Chim. et Phys., s. 5, t. 2, p. 289-324. 1874.

—— TRAITÉ DE MICROBIOLOGIE. t. 3, p. 388. Paris, 1900.

[3] GILLESPIE, L. J., and WALTERS, E. H. THE POSSIBILITIES AND LIMITATIONS OF THE DUCLAUX METHOD FOR THE ESTIMATION OF VOLATILE ACIDS. *In* Jour. Amer. Chem. Soc., v. 39, no. 9, p. 2027-2055, 3 fig. 1917. Literature cited, p. 2055.

[4] DYER, D. C. A NEW METHOD OF STEAM DISTILLATION FOR THE DETERMINATION OF THE VOLATILE FATTY ACIDS, INCLUDING A SERIES OF COLORIMETRIC QUALITATIVE REACTIONS FOR THEIR IDENTIFICATION. *In* Jour. Biol. Chem., v. 28, no. 2, p. 445-473, 2 fig. 1917.

TABLE III.—*Acidity of 100 gm. sugar beet top silage containing moisture*

| Sample No. | Moisture. | Dry material. | Acetic acid. | Propionic acid. | Butyric acid. | Valeric acid. | Total volatile acid. | Lactic acid. | Total acids. |
|---|---|---|---|---|---|---|---|---|---|
| | *Per cent.* | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | 81.5 | 18.5 | 0.51 | 0 | 0.73 | 0 | 1.24 | 0.59 | 1.83 |
| 2 | 76.0 | 24.0 | .42 | 0 | .17 | 0 | .59 | .69 | 1.28 |
| 3 | 49.5 | 50.5 | .17 | 0.15 | .25 | 0 | .57 | Trace. | .57 |
| 5 | 69.5 | 30.5 | .71 | .05 | 1.13 | 0 | 1.89 | 1.71 | 3.60 |
| 6 | 70.0 | 30.0 | 0 | 0 | .54 | 0.04 | .58 | Trace. | .58 |
| 7 | 70.0 | 30.0 | .29 | .10 | 0 | 0 | .39 | .41 | .80 |
| 8 | 78.5 | 21.5 | .31 | .05 | .44 | 0 | .80 | .26 | 1.06 |
| 9 | | | | | | | | | |

TABLE IV.—*Acidity of 100 gm. sugar beet top silage on dry basis*

| Sample No. | Acetic acid. | Propionic acid. | Butyric acid. | Valeric acid. | Total volatile acid. | Lactic acid. | Total acids. |
|---|---|---|---|---|---|---|---|
| | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | 2.73 | 0 | 3.92 | 0 | 6.65 | 3.07 | 9.72 |
| 2 | 1.74 | 0 | .70 | 0 | 2.44 | 2.79 | 5.23 |
| 3 | .35 | 0.29 | .50 | 0 | 1.14 | Trace. | 1.14 |
| 6 | 2.32 | .17 | 3.70 | 0 | 6.19 | 5.61 | 11.80 |
| 7 | 0 | 0 | 1.79 | 0.12 | 1.91 | Trace. | 1.91 |
| 8 | .96 | .34 | 0 | 0 | 1.30 | 1.35 | 2.65 |
| 9 | 1.46 | .25 | 2.04 | 0 | 3.75 | 1.21 | 4.96 |

In the samples of sugar beet top silage, sample 8 is the only one that contains the same acids that are found in corn silage. This silage was classed as a fair quality of silage by experts when it was sent to this station. The remaining samples of silage all contained some butyric acid. The quality of the silage ranged from fair to poor, depending chiefly upon the amount of dirt that was in the silage. While the amount of butyric acid present indicates in a degree the type of fermentation, it does not seem to prevent stock from eating the silage. Some samples contained butyric acid in quantities that made drying the material in an oven very unpleasant unless the process was carried on under a hood, and yet cattle ate the silage with relish. It is not known how much effect the abnormal fermentation has on the feeding value of silage, but no doubt some loss occurs. Such losses could be greatly reduced by carefully packing the beet tops when siloing and by covering the tops in such manner that all the air is excluded.

The mere presence of butyric acid in silage is not in itself harmful, but it is the fact that the presence of butyric acid indicates an abnormal fermentation, resulting in a partial decomposition of silage, which tends to lower its feeding value.

It is hardly to be expected that beet tops can be packed sufficiently to exclude all air, because of the nature of the tops, but possibly cutting the tops in a silage cutter would solve the problem. Experiments are planned for the coming year to determine the best methods of siloing sugar beet tops.

Lactic acid is present in very small amounts in many of the samples. It is possible that more lactic acid is present in the early stages of fermentation and that it is either changed into other acids or is decomposed. An additional investigation is needed to explain fully the reason for the small amounts of lactic acid in abnormal silage. The lactic acid present is the racemic mixture.

The fact that sample 8 contains the characteristic acids of normal silage indicates that sugar beet tops can be successfully siloed if proper precautions are taken to pack the tops well and exclude air. The samples of silage analyzed came from a pit silo ranging from 2½ feet to 8 feet in depth. Without question, depth of the pit silo is an important factor in the production of good silage. Where shallow silos are used, air gains access to the greater portion of the beet tops and a poor silage results, whereas, in the deeper silos there is less chance for the entire silage to be partially spoiled on account of access of air. It is important, then, to have a deep silo to eliminate dirt, and to pack thoroughly so as to exclude air. These precautions will insure a better average silage throughout the Northwest than is now found.

## SUMMARY

(1) It is evident that the quality of sugar beet top silage put up by the average Idaho farmer is very poor.

(2) Large quantities of dirt are present, which could be eliminated in a large measure by careful handling of the product during siloing.

(3) To improve the quality of silage, pit silos should be deep and the silage should be packed thoroughly and covered sufficiently to exclude air. Excess dirt should be eliminated.

(4) More care should be taken by the average farmer in siloing sugar beet tops. While stock will eat silage that is very poor, there is a loss of food value in improperly made silage as well as danger of mortality.

# NODULE BACTERIA OF LEGUMINOUS PLANTS

By F. LÖHNIS, *Soil Biologist, Bureau of Plant Industry, United States Department of Agriculture*, and ROY HANSEN, *Professor of Soils, University of Saskatchewan, Saskatoon, Sask.*[1]

## INTRODUCTION

Despite the fact that the nodule bacteria of the leguminous plants have been made the subject of numerous publications, it is not to be disputed that their true morphological and physiological character, as well as their correct systematic position, are by no means sufficiently known. This is especially clearly demonstrated by the fact that they are still proclaimed by several writers to be the representatives of a special genus Rhizobium, once established by A. B. Frank as the result of rather inadequate studies upon this subject. In the new classification of bacteria, adopted by the Society of American Bacteriologists, the nodule bacteria again are widely separated from closely related species, and the error concerning the so-called genus Rhizobium has been revived once more.

Comparative investigations upon the symbiotic and the nonsymbiotic nitrogen-fixing bacteria of the soil, published in 1905 by the senior author, have proved conclusively that the nodule bacteria are not representatives of a special genus Rhizobium, but that they are closely related to *Bacillus radiobacter* Beijerinck and further to *B. lactis viscosum* Adametz, *B. pneumoniae* Friedländer, and *B. aerogenes* Escherich. The last three organisms are immotile, while the first one is motile; but here again the very close relationship between the immotile *B. aerogenes* and the motile *B. coli* has to be kept in mind. In fact, there can be easily isolated from every soil numerous varieties of *B. radiobacter*, which lead gradually up to *B. coli*, acquiring the power of fermentation and that type of growth on solid substrates which is characteristic of the last-named species. It has been pointed out in detail that all species mentioned above differ only gradually, not principally, in their physiological and morphological qualities, and especially that those branched or otherwise changed cell forms which are frequent in the root nodules are equally common with all members of this group of capsule bacteria, if these are tested adequately.[2] The ability to fix the atmospheric nitrogen was shown to be common in this group of organisms.

[1] Most of the experiments discussed in this paper were made in the summer of 1919, at the University of Illinois, where at that time the junior author held the position of Associate in Soil Biology. The photographs accompanying the paper were made by Mr. F. L. Goll, of the Bureau of Plant Industry, United States Department of Agriculture.

[2] It is not superfluous to emphasize once more that persistence in calling these forms "bacteroids" is by no means to be recommended. They are true bacteria, not foreign bodies looking like bacteria, as Frank's pupil Brunchorst erroneously believed. To speak of a "bacteroid" growth of bacteria is no less absurd than it would be to speak of a "fungoid" growth of fungi.

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*Bacillus radiobacter* was found to be peritrichic, and the same paper also indicated (*12, p. 592, footnote*)[1] that in all probability *B. radicicola* has the same kind of flagellation. But no faultless preparates were obtained at that time.

In the same year, 1905, G. T. Moore wrote concerning the nodule bacteria (*14, p. 26*):

> There does not seem to be any necessity for creating a new group to include these organisms, as has been done by Frank, under the name of Rhizobium, for although there is a certain amount of polymorphism, it is no greater than frequently occurs in the bacteria.

With regard to the flagellation, however, Moore himself evidently made no special studies, and, accepting Beijerinck's statement that the "swarming bodies" (gonidia) of *Bacillus radicicola* are monotrichic as being valid for the bacteria too, he proposed to call the nodule bacteria *Pseudomonas radicicola*. Numerous authors have followed this suggestion, and experiments made by Harrison and Barlow (*8*) apparently confirmed the view that the flagellation of these organisms is indeed monotrichic.

However, these experiments are, in fact, not convincing, as has been emphasized especially by Kellerman (*9*). This author and also G. de Rossi (*16, 17*), Zipfel (*19*), and Prucha (*15*) secured results all of which demonstrated more or less clearly that the senior author's assumption was correct: *Bacillus radicicola* is peritrichic; it is no "Pseudomonas."

But this seemed again to be contradicted by certain results obtained by the junior author while working with the late T. J. Burrill (*6*). Numerous tests made with the bacteria isolated from cowpea, soybean, Japan clover, and other plants showed clearly and invariably monotrichic flagellation, and, therefore, the designation *Pseudomonas radicicola* was restored once more. Additional results, however, indicated that there are other features which differentiate the bacteria of the cowpea-soybean group from those living in the roots of clover, alfalfa, pea, and vetch. Especially the slime production and the speed of growth appeared to be different, and the organisms studied were arranged into two groups, "slow growers" and "fast growers" Both, however, were supposed to be merely varieties of *P. radicicola*.

This point remained to be investigated more thoroughly. In addition, another "fast grower" presented itself for detailed study, which quite regularly appeared on thickly sown plates of the "slow growing" groups, and which, indeed, has been mistaken by several investigators as the true nodule organism of cowpea, soybean, Japan clover, etc. Repeatedly such cultures were sent to and tested by the junior author. They were all unable to produce nodules.

The data given on the following pages make it evident that this "fast grower" is *Bacillus radiobacter*, which plays in this case, also, a very

[1] Reference is made by number (italic) to "Literature cited," p. 554-555.

interesting rôle. As this same species undoubtedly takes part in many processes occurring in soil and in water, it was thought useful to give another more detailed description of it, especially because, despite its ubiquity, *B. radiobacter* is much too little known. In addition to the rather short description given by Beijerinck, only the more complete one published by the senior author in 1905 exists thus far. On account of its great similarity to *B. radicicola*, *B. radiobacter* should be very well known to all bacteriologists working with the nodule bacteria in order to avoid mistakes which may otherwise not be discovered until only negative results are obtained in the inoculation tests.

Concerning the flagellation of the nodule bacteria three statements have been published more recently which also will have to be discussed presently. According to J. K. Wilson (*18*) the soybean bacteria are peritrichous; Barthel (*2*) declared lupine and alfalfa bacteria to be lophotrichous; Fred and Davenport (7) found the alfalfa organism peritrichous, but they found the lupine bacteria characterized by having one, rarely two, flagella.

## EXPERIMENTAL RESULTS

The following strains of nodule bacteria were studied after having been tested with positive results in regard to their ability to produce nodules on the host plants from which they were isolated.

1. Cowpea.
2. Peanut.
3. Japan clover.
4. Beggar weed.
5. Soybean.
6. Red clover.
7. Sweet clover.
8. Vetch.
9. Strophostyles.

There were also included in our investigations two strains isolated from:

10. Black locust.
11. Lupine.

No positive inoculation test could be made on black locust. The lupine culture was kindly furnished by Dr. E. B. Fred, of the University of Wisconsin, who had tried it with positive results on this plant. Our tests were equally successful.

Two noninfectious "fast growing" cultures isolated from legume nodules and identified as *Bacillus radiobacter* were studied in comparison with six Radiobacter strains which originated from soil and which were kept in the senior author's collection since the years given in parentheses.

12. Fast grower from cowpea.
13. Fast grower from soybean.
14. *Bacillus radiobacter* from soil (1904).
15. Same (1907).
16. *Bacillus radiobacter* from soil (1908).
17. Same (1908).
18. Same (1908).
19. Same (1916).

No. 14 is the strain which in 1904 had been acknowledged by Prof. Beijerinck as being identical with his *Bacillus radiobacter* and which was used by the senior author for the original description published in 1905 (*12*).

TABLE I.—*Development of cowpea-soybean bacteria, Bacillus radicicola (from clover, vetch, etc.), and B. radiobacter*

| Substrates. | Cowpea-soybean bacteria. |
|---|---|
| Mannite-nitrate agar slant. | MACROSCOPIC EXAMINATION.—Raised whitish to porcelain white, glossy layer.<br>MICROSCOPIC EXAMINATION.—After 3 days slender rods, sometimes curved; after 7 to 10 days unstained, irregular sheaths, with 1 to 4, most frequently 2, darkly stained granules; after 2 to 3 weeks many small globules, ovals, and short rods outside of the unstained sheaths, also small globular regenerative bodies. |
| Beef agar slant. | MACROSCOPIC EXAMINATION.—Fairly good whitish growth.<br>MICROSCOPIC EXAMINATION.—After 3 days weakly stained, irregular, thin, short rods; after 7 to 10 days irregular rods, producing gonidia and globular regenerative bodies, which may multiply as such; after 2 to 3 weeks very variable appearance, rather long slender rods, often branched, or club-shaped, globular regenerative bodies, also unstained, irregular sheaths with dark granules, and large globular gonidangia. |
| Beef gelatin stab. | MACROSCOPIC EXAMINATION.—Very small, gray, nonliquefying disk on the surface, hardly any growth in the stab.<br>MICROSCOPIC EXAMINATION.—Thin rods, sometimes branched or swollen, producing gonidia and small globular regenerative bodies; in old cultures gonidia and regenerative bodies frequently predominating. |
| Beef broth. | MACROSCOPIC EXAMINATION.—Broth at first clear, with little sediment; later (after about 2 weeks) slightly turbid.<br>MICROSCOPIC EXAMINATION.—After 3 days slender rods, sometimes curved; after 2 weeks granular rods producing gonidia, also budding and branching, small globular regenerative bodies, and symplasm; after 3 to 4 weeks very irregular forms, branching, swelling. |
| Milk. | MACROSCOPIC EXAMINATION.—During the first weeks no change visible, later slow digestion, no clear serum zone.<br>MICROSCOPIC EXAMINATION.—Mostly small globules and ovals, few short, slender rods. |
| Potato. | MACROSCOPIC EXAMINATION.—Very meager, transparent, thin layer.<br>MICROSCOPIC EXAMINATION.—After 7 days slender rods, sometimes branched, or with terminal swelling; after 4 weeks small globules and ovals, irregular rods (frequently branched), globular regenerative bodies, and symplasm with very variable new development. |

TABLE I.—*Development of cowpea-soybean bacteria, Bacillus radicicola (from clover, vetch, etc.), and B. radiobacter*—Continued

| Substrates. | *B. radicicola* (from clover, vetch, etc.). | *B. radiobacter.* |
|---|---|---|
| Mannite-nitrate agar slant. | MACROSCOPIC EXAMINATION.—Slimy, transparent growth, with or without whitish streaks.<br>MICROSCOPIC EXAMINATION.—Small ovals and short rods, producing after 1 to 2 weeks gonidia and small globular regenerative bodies. Also unstained slime threads with dark granules and large globular, or oval gonidangia; irregular pale forms from symplasm. | MACROSCOPIC EXAMINATION.—Thick, slimy transparent layer, with whitish streaks.<br>MICROSCOPIC EXAMINATIONS.—After 7 days small ovals and short rods, imbedded in slime; after 14 days some rods with thick unstained capsules forming symplasm; after 3 to 4 weeks normal cells, stars, and large globules and clubs from symplasm. |
| Beef agar slant. | MACROSCOPIC EXAMINATION.—Meager, flat, grayish growth.<br>MICROSCOPIC EXAMINATION.—Mostly small ovals and short rods, the latter sometimes curved, budding and branching; later also large rods, and large globular, oval, or club-shaped gonidangia. | MACROSCOPIC EXAMINATION.—Flat, whitish slimy layer, thick sediment below.<br>MICROSCOPIC EXAMINATION.—As on mannite-nitrate agar. |
| Beef gelatin stab. | MACROSCOPIC EXAMINATION.—Small, gray, nonliquefying disk on surface, very little growth in stab.<br>MICROSCOPIC EXAMINATION.—Small ovals and short rods, gonidia, and small globular regenerative bodies. | MACROSCOPIC EXAMINATION.—Grayish, flat, round, nonliquefying surface growth, little growth in stab; after 2 to 4 weeks gelatine sometimes brown on top.<br>MICROSCOPIC EXAMINATION.—Typical ovals and short rods, these sometimes curved or branched, also unstained slime threads with dark granules, later symplasm with stars |
| Beef broth. | MACROSCOPIC EXAMINATION.—Broth either clear or very slightly turbid, little whitish sediment.<br>MICROSCOPIC EXAMINATION.—Small ovals and short rods, budding and branching, occasionally threads; after 1 to 2 weeks many gonidia and small, globular regenerative bodies. | MACROSCOPIC EXAMINATION.—Broth turbid, white ring, whitish film, much whitish sediment.<br>MICROSCOPIC EXAMINATION.—Small ovals and short rods, budding and branching; later gonidia, globular regenerative bodies, threads, and fine stars from symplasm. |
| Milk. | MACROSCOPIC EXAMINATION.—After 1 to 4 weeks clear serum zone on top, 2 to 5 mm. thick; milk below nearly unchanged, very fine flocculation.<br>MICROSCOPIC EXAMINATION.—Small ovals and rods, later also granular threads and symplasm. | MACROSCOPIC EXAMINATION.—First slime ring and serum zone on top; later whole milk turning brown.<br>MICROSCOPIC EXAMINATION.—After 7 days typical ovals and rods; later small and large cells from symplasm. |
| Potato. | MACROSCOPIC EXAMINATION.—Meager, transparent, slimy growth.<br>MICROSCOPIC EXAMINATION.—Small slender rods, budding and branching, some ovals, globular regenerative bodies; later gonidia predominant. | MACROSCOPIC EXAMINATION.—First gray, later coli-brown slimy layer, potato turns frequently gray.<br>MICROSCOPIC EXAMINATION.—First small ovals and short rods, budding and branching, later also large oval and globular gonidangia and symplasm with stars. |

The results of our investigations leave no doubt that the earlier findings of the junior author were correct so far as the polar flagellation and the peculiar morphological and cultural features of the cowpea-soybean organisms are concerned. On the other hand, it could now be ascertained with equal certainty that the bacteria producing nodules on clover, alfalfa, vetch, and other plants originally cultivated in Europe are all peritrichic and exhibit all the characteristics of *Bacillus radicicola*, as described by Beijerinck and other authors.

Those findings which were obtained most frequently and which may be considered as being typical for the two groups of nodule bacteria are compiled in Table I, together with analogous data pertaining to *Bacillus radiobacter*. Photographs of the most characteristic details are reproduced on Plates 68 and 69.

When grown from the root nodule on Harrison and Barlow's ash agar, mannite agar, or similar agar of low nitrogen content, the two groups of nodule bacteria are best characterized and differentiated by the very slow growth of colonies in the cowpea-soybean group and the comparatively quick growth of those of *Bacillus radicicola* (*6, pl. 11, fig. 1-11*). Frequently, but not always, the development of *B. radiobacter* is still somewhat more rapid than that of *B. radicicola;* in the macroscopical as well as in the microscopical aspects, however, the colonies of these two species on such media are so very much alike that it is almost impossible to distinguish them with certainty. Both, when developing on the surface, are perfectly round, drop-like, soft, watery or slimy, glistening, transparent. Often a whitish center or whitish streaks become visible within the more transparent mass, especially if the surface colony is the outgrowth of an imbedded colony. Sometimes it appears as if this whitish center were regularly to be seen only with certain strains of Radicicola and Radiobacter. This is not the case, however. Its presence or absence is erratic and can not be used for differentiation. The imbedded colonies are always small, white, opaque, mostly lentiform, less frequently circular. Under the microscope the surface colonies present themselves as sharp-edged disks, pure white at the outside with yellowish-grayish granulation in the center. In a few cases a radiate structure becomes visible. The colonies of the cowpea-soybean group appear macroscopically, as well as microscopically like young colonies of the Radicicola type. The presence of Radiobacter colonies on the plate stimulates their growth markedly.

In cell morphology there is again a more pronounced relationship between Radiobacter and Radicicola than between the nodule bacteria of the clover-vetch group on the one side and of the cowpea-soybean group on the other. This holds true with the regular rod forms as well as with the very pleomorphic, curved, swollen, branched, or otherwise changed types of growth characteristic of these groups. The photographs on Plate 68, D–L, represent the pictures we have seen most frequently, but they do not pretend to give a complete illustration of the wide pleomor-

phism of these organisms. Before their complete life history can be given much additional material will have to be collected, especially with regard to the form of gonidangia, regenerative bodies, and the various cells developing from the symplastic stage. At present we intend only to bring out as clearly as possible those points which are important for a correct differentiation and diagnosis. As far as one may judge from the microscopic appearance, it is the inclination of *Bacillus radiobacter* to form stars which is most characteristic (Pl. 68, L), and this was used, therefore, by Beijerinck for its denomination. With *B. radicicola* the frequent occurrence of the clear-cut, compact Y forms is the most conspicuous feature (Pl. 68, H); whereas the bacteria of the cowpea-soybean group present themselves in most cases, when stained with aqueous aniline dyes in the usual manner, as short or long, unstained sheaths with one or more darkly stained granules (Pl. 68, J). Of course Y forms, as well as unstained sheaths with darkly stained gonidia, can be observed not infrequently with the other organisms, too, and the star formation is by no means solely to be found with Radiobacter; but we feel sure that those pictures, as shown on Plate 68, G–L, will be found most valuable for diagnostical purposes.

The flagellation is the same with Radiobacter (Pl. 68, C) and Radicicola (Pl. 68, B), while the bacteria of the cowpea-soybean group are characterized by one coarse, fairly straight polar flagellum (Pl. 68, A). Just before fission one cilium may be seen at each end; as a rare exception a tuft of polar flagella was observed occasionally. Frequently a darkly stained body becomes visible within the rod just at that point where the flagellum springs forth, which may be considered to be a flagellated, not yet liberated, gonidium, such as can be seen occasionally with many other bacteria, especially with *Bacillus radicicola*, too. When liberated this becomes the monotrichic small "swarming body" described by Beijerinck in 1888 (*4*).

The growth on mannite-nitrate agar, as well as on beef agar slants, as described in Table I, is quite characteristic, and after the eyes have been sufficiently trained, one seldom makes a mistake in guessing the group to which a culture presented for inspection may belong. But it must be admitted that occasionally and temporarily a strain of the cowpea-soybean group can show the flat, transparent growth characteristic of Radicicola, whereas it is a very rare occurrence that a member of the last-named group simulates the former one. The growth of Radiobacter is always very typical, except when a very weak strain is encountered, which does not frequently occur within this group. Plate 69, A, demonstrates the characteristic differences noticeable on mannite-nitrate agar as clearly as they can be shown in a photographic reproduction.[1]

[1] As was the case with Azotobacter, for which the mannite-nitrate agar was first used (*13, p. 686*), so also the nodule bacteria and *Bacillus radiobacter* grew very readily on this substrate. Allen (*1, p. 33*) asserted recently that he could not get any growth of Azotobacter on a dextrose agar, which he erroneously called "Löhnis and Smith's medium." But not even the formula used by us has been quoted correctly by Allen, and it is, of course, quite obvious that on account of the alterations made by Allen his agar must indeed have been quite unsuitable.

Cultures on beef gelatine and in beef broth differentiate clearly Radiobacter and nodule bacteria, while, as stated in Table I, the two groups of nodule organisms grow very much alike on these substrates. Microscopic tests, however, made from gelatine and broth furnish, in most cases, especially characteristic pictures, provided that the growth has not been altogether too poor to get a satisfactory preparate.

The growth in milk and on potato, as described in Table I and illustrated on Plate 69, is very characteristic and can be used to great advantage for diagnosis. It is not to be denied that with old stock cultures atypical results may sometimes be obtained in this direction also. Especially cultures rich in or entirely made up of the globular regenerative bodies, which are produced by these as well as by all other bacteria, furnish whitish, yellowish, or only slightly brownish growth on potato in the case of *Bacillus radiobacter* and *B. radicicola*. But we have never seen such atypical growth with new isolations. Here the coli-brown color of the potato cultures separates Radiobacter sharply from the nodule bacteria, and these in turn are equally sharply to be distinguished by the behavior of their milk cultures. It is true that sometimes milk cultures of the *B. radicocola* group also leave the milk unchanged, but the microscopic test of such abnormal cases probably will always show, as it did in the cases studied by us, that the abnormality was simply caused by the fact that the bacteria which were inoculated did not multiply at all. Furthermore, no alteration may be seen if milk is used which has been kept for a long time and has become concentrated by evaporation of part of its water.

To determine on a larger scale whether this different behavior of the two groups of nodule bacteria, when grown in milk, can be correctly accepted as of real diagnostic value, all cultures of nodule bacteria at our disposal were tested simultaneously with the following results:

| MILK WAS CHANGED AS TYPICAL FOR BACILLUS RADICICOLA BY THE FOLLOWING CULTURES: | MILK WAS LEFT UNCHANGED BY THE FOLLOWING CULTURES: |
|---|---|
| 5 from red clover. | 10 from cowpea. |
| 4 from sweet clover. | 8 from soybean. |
| 6 from navy bean. | 5 from peanut. |
| 1 from vetch. | 4 from Japan clover. |
| 2 from lupine. | 2 from beggar weed. |
| 3 from black locust. | 2 from *Cassia chamaecrista*. |
| 3 from Amorpha. | |
| 2 from Strophostyles. | |

If kept for longer than four weeks milk cultures of the cowpea-soybean organisms usually become more or less transparent on account of partial decomposition of the casein; but they never show the perfectly clear zone characteristic of the other group.

The bacteria were also tested on other media besides the standard substrates, of which sterilized soil, moistened with 0.5 per cent mannite

solution, mannite-nitrate solution as used for studying the life cycle of Azotobacter, tap water plus 0.5 per cent beef broth, and 2 per cent salt agar furnished the most satisfactory results, especially with regard to a more complete knowledge of the cell morphology of the organisms. For diagnostic purposes, however, these substrates are of minor importance, as they do not bring out anything which is not already to be seen on the standard media. Nevertheless, it should be pointed out that cultures of the nodule bacteria in soil are to be recommended for two reasons. First, they are useful in keeping the organisms in a normal state of virility for a long time, and, in the second place, they demonstrate very clearly, when studied microscopically, that it is erroneous to believe—though numerous authors have promoted such hypotheses—that the nodule bacteria behave very differently in soil and could, therefore, not be isolated in their typical form from their natural habitat. Our results are in complete agreement with those recently obtained by Barthel (3) concerning the growth of bacteria in sterilized soil.

Tap water containing 0.5 per cent beef broth gave also very good development and proved repeatedly helpful in reviving old, weakened strains which refused to grow on solid substrates.

## DISCUSSION

Our experimental results leave no doubt that the nodule bacteria of the leguminous plants are to be divided at least into two distinct groups, differing morphologically as well as culturally. It is equally beyond dispute that these differences are so marked and constant that one might be inclined to establish the nodule organism of the cowpea-soybean group as a new species. On account of its behavior in the inoculation test O. Kirchner has considered the soybean organism a distinct species, which he named in 1895 *Rhizobacterium japonicum*(10). According to the rules of priority, this species name would have to be given preference, despite the fact that different behavior in the inoculation test generally can not be accepted as a distinguishing mark of such quality as to validate the creation of a new species. The generic name Rhizobacterium, used by Kirchner, is, of course, equally as untenable as the generic name Rhizobium. According to the two most requently used modes of classifying the bacteria, one might name the cephalotrichic non-sporulating rod of the cowpea-soybean group *Pseudomonas japonica* or *Bacterium japonicum*, while the name *Bacterium* or *Bacillus radicicola* would have to be retained for the peritrichic organisms to be found with clover, alfalfa, sweet clover, vetch, pea, etc.

However, we do not advocate such a procedure. We are firmly of the opinion that much more must be known of the complete life history of a bacterium than is obtainable along the standardized lines of customary bacteriological research, before one can safely proceed to establish a genuine species on a truly scientific basis. Undoubtedly many if

not most of the commonly used so-called species names of bacteria are no species names at all, but merely denominations more or less correctly applied to organisms about whose complete life history and, accordingly, about whose true systematic value and position comparatively little is known at present.

It is by no means impossible that future systematic investigations may demonstrate the peritrichic and the cephalotrichic nodule bacteria to be relatively constant types of growth of one species. There are a few reports in the literature indicating that occasionally cross inoculations have been obtained, which might support this hypothesis. While O. Kirchner never found nodules on soybeans grown in Germany and therefore thought his *Rhizobacterium japonicum* to be the active agent in the Far East, F. Cohn said in a note appended to Kirchner's report that soybeans grown for the first time in his experimental garden in Breslau did produce nodules, though these were not of the normal type and contained only a few rodlike bacteria. Kellerman reported upon a case where a culture originally isolated from alfalfa was found to be infective on alfalfa and lupine as well as on soja when tested by Leonard after six years' cultivation on artificial substrates. It may be mentioned also in this respect that cross inoculations between navy bean and cowpea seem to be equally possible, under circumstances, however, which need further elucidation.

But just as negative results in cross inoculation experiments can not be accepted as sufficient basis for establishing different species, so also such rather exceptional positive results can not be used as valid support of the hypothesis that monotrichic and peritrichic nodule bacteria are only two types of growth of the same species. First of all, it would have to be ascertained whether in such cases the peritrichic organism has really changed into the monotrichic one, or vice versa. The possibility remains, of course, that occasionally the one type of organisms may invade a host plant whose nodules are normally caused by the other type of bacteria.

Changes in flagellation from peritrichic to cephalotrichic have been observed, according to Lehmann and Neumann (*11, p. 256, 357, 371*), with *Bacillus coli* and *B. alcaligenes*. Both species are related to *B. radiobacter* and *B. radicicola*, and under this aspect an analogous change should not be rejected prematurely as *a priori* improbable.

At the end of the introduction three statements have been quoted from the more recent literature which one might be inclined to accept as confirmative evidence in this direction. However, on account of the following reasons we do not feel justified in advocating such an interpretation.

J. K. Wilson says that in his preparations of soybean organism—

> The flagella were peritrichous, the highest number found being four.

As no photomicrographs had been made, Dr. Wilson was kind enough to furnish, on request of the senior author, one of his slides for examina-

tion. The flagella visible therein were all very weakly stained, so that no definite conclusion could be drawn. A culture, for which we are also indebted to Dr. Wilson, behaved in our hands like all those tested before; practically all cells were distinctly monotrichous. A comparison of Plate 68, A, with the pictures published on Plates IV and V of Bulletin 202, Illinois Agricultural Experiment Station (*6*), leaves no doubt about this point.

In Barthel's paper (*2*, *p. 16*) two drawings and one photomicrograph are to be found which clearly illustrate the following statement:

Bei den Lupinenbakterien sind die Geisseln ziemlich lang, wellig geformt und an einem Pole befestigt. Ihre Anzahl variiert von 1 bis 6. Ihre Placierung ist recht eigentümlich. Sie sitzen nämlich öfters nicht gerade an der Spitze des Zellleibes, sondern sozusagen an den "Ecken" und oft etwas von dem Hinterende entfernt. Oft findet man auch eine Geissel an der einen "Hinterecke" und mehrere andere zusammen an der anderen. . .

Bei den Luzernebakterien waren die Geisseln meist weniger und kürzer, am häufigsten 1 oder, seltener 3 oder 4, aber auch hier deutlich lophotrich. . .

Fred and Davenport (7), on the other hand, saw only one or two cilia with the lupine bacteria, while several strains of alfalfa organisms left no doubt as to their peritrichic flagellation.

We believe that these conflicting views are in fact not so irreconcilable as they seem to be. If well-made slides are examined carefully, some cells will always be discovered which clearly show that on account of the primary swelling and the following shrinking of their capsules, the flagella are often more or less dislocated. Some of the cells shown in Plate 68, A-C, exhibit this phase as clearly as it is possible in such reproductions. The flagella of the monotrichous bacteria of the cowpea-soybean group are to be seen in an exactly polar position only when the cells themselves are lying lengthwise within the "drift," as indicated by the floating flagella. In all other cases dislocations may take place, removing the cilia to the corners or even to the side of the cells, where they should not be viewed, however, as remnants of a peritrichic flagellation.

On the other hand, analogous disturbances may cause the occurrence of apparently cephalotrichic bacteria among the peritrichic cells of *Bacillus radicicola* and *B. radiobacter*. That there exists no truly polar flagellation in these cases, however, is evidenced by the fact that the cilia composing such an apparently polar tuft do not protrude exactly from the same spot, as they do, for example, in the cell with several polar flagella shown in Plate 68, A. They are always more or less separated and are only accidentally drawn together in the course of the shrinking of the capsule. A thorough examination of well-made preparations leaves no doubt that the original position of the flagella is peritrichic.

## SUMMARY

(1) The nodule bacteria of the leguminous plants are to be divided into two groups, differing morphologically as well as physiologically.

(2) The first group shows all features characteristic of *Bacillus radicicola* Beijerinck. It is peritrichic, grows relatively fast on agar plates, and changes the milk in a very characteristic manner. It produces nodules on the roots of the following plants: clover, sweet clover, alfalfa, vetch, pea, navy bean, lupine, black locust, Amorpha, and Strophostyles.

(3) The second group is characterized by monotrichic flagellation, comparatively very slow growth on agar plates, and inability to cause a marked change in milk. It has been isolated from cowpea, soybean, peanut, beggarweed, Acacia, Genista, and Cassia.

(4) According to the customary manner of classifying bacteria, this second group of nodule bacteria would have to be considered to be a new species, and according to the rules of priority, it would have to be named *Pseudomonas japonica* or *Bacterium japonicum* (Kirchner). But we do not advocate such a procedure, because only a complete study of the life history of these two groups of organisms would make it possible to say definitely whether they are, indeed, two distinct species or merely different types of growth of the same organism.

(5) *Bacillus radicicola* is closely related to *B. radiobacter*. The generic name Rhizobium is to be rejected. The correct systematic position of both species is in the neighborhood of *B. aerogenes* and *B. coli*.

(6) *Bacillus radiobacter* seems to be regularly present in the root nodules of leguminous plants, stimulating development and activity of the nodule bacteria. On account of its similarity to *B. radicicola*, it has been repeatedly mistaken for the nodule-producing organism in the cowpea-soybean group, whose bacteria it outranks very considerably in the development on the plates made from the nodules. By its brown growth on potato, *B. radiobacter* can be easily differentiated from *B. radicicola*.

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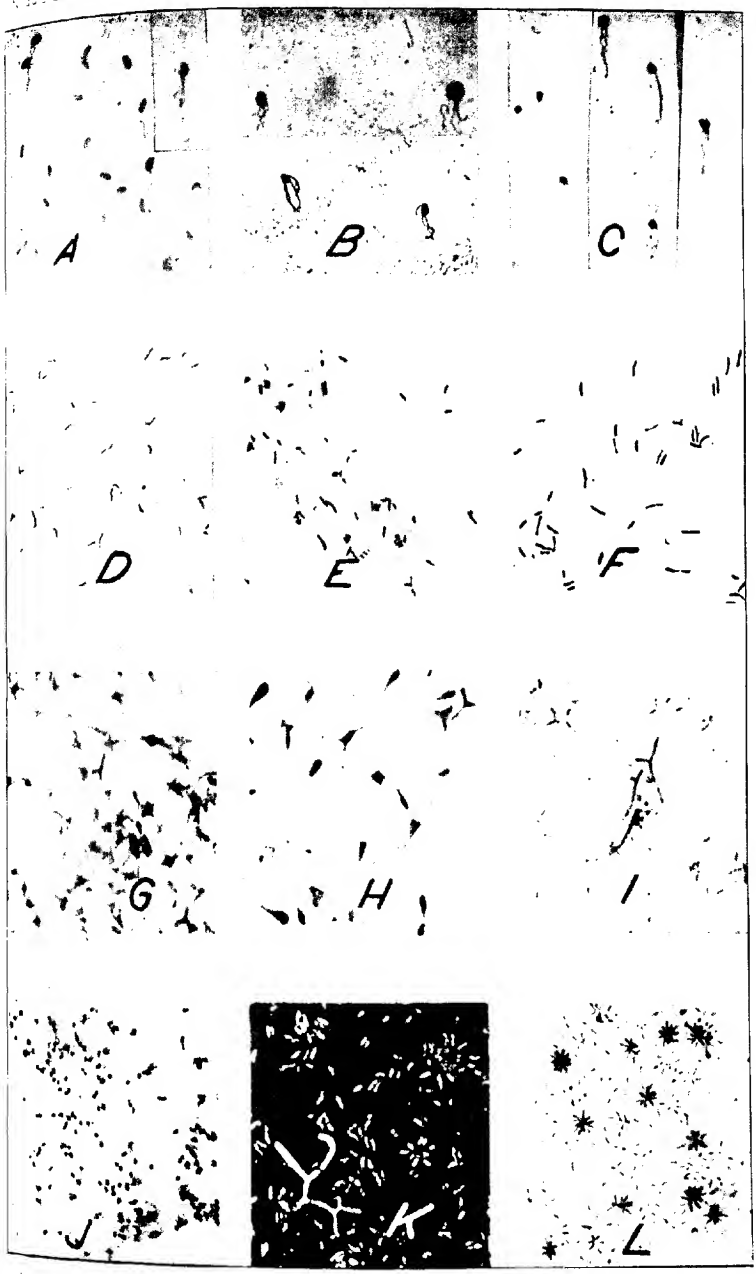
PLATE 68

A.—Soybean bacteria, J. K. Wilson's strain, 4 days old.
B.—Vetch bacteria, 3 days old.
C.—Bacillus radiobacter, 2 days old.
D.—Soybean bacteria, beef agar, 4 days old.
E.—Red clover bacteria, beef agar, 4 days old.
F.—*Bacillus radiobacter*, beef agar, 4 days old.
G.—Cowpea bacteria, potato, 6 days old.
H.—Red clover bacteria, potato, 14 days old.
I.—*B. radiobacter*, milk, 7 days old.
J.—Cowpea bacteria, mannite-nitrate agar, 8 days old.
K.—Vetch bacteria, mannite-nitrate agar, 8 days old.
L.—*B. radiobacter*, mannite-nitrate solution, 17 days old.
A–C Loeffler's stain; D–L aqueous fuchsin. ×1,000.


A
B
C
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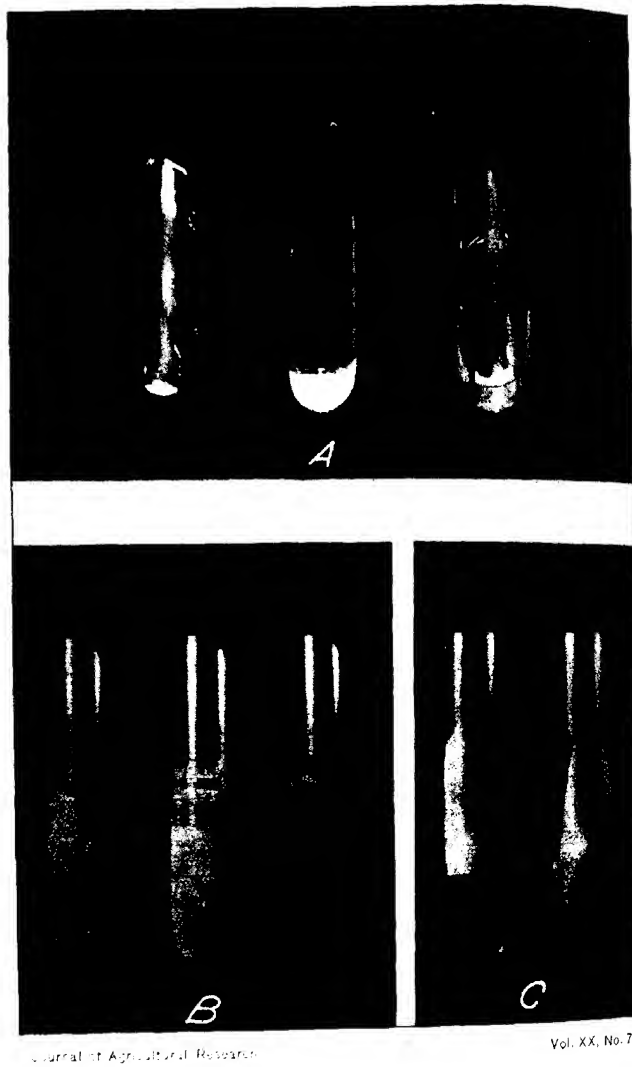
A
B
C

PLATE 69

A.—Mannite-nitrate agar slants, 8 days old, from left to right: soybean bacteria, vetch bacteria, and *Bacillus radiobacter*.

B.—Growth in milk, 4 weeks old from left to right: soybean bacteria, vetch bacteria, and *B. radiobacter*.

C.—Growth on potato, 2 weeks old: vetch bacteria (left) and *B. radiobacter* (right).

# CORRELATION AND CAUSATION

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## PART I. METHOD OF PATH COEFFICIENTS

### INTRODUCTION

The ideal method of science is the study of the direct influence of one condition on another in experiments in which all other possible causes of variation are eliminated. Unfortunately, causes of variation often seem to be beyond control. In the biological sciences, especially, one often has to deal with a group of characteristics or conditions which are correlated because of a complex of interacting, uncontrollable, and often obscure causes. The degree of correlation between two variables can be calculated by well-known methods, but when it is found it gives merely the resultant of all connecting paths of influence.

The present paper is an attempt to present a method of measuring the direct influence along each separate path in such a system and thus of finding the degree to which variation of a given effect is determined by each particular cause. The method depends on the combination of knowledge of the degrees of correlation among the variables in a system with such knowledge as may be possessed of the causal relations. In cases in which the causal relations are uncertain the method can be used to find the logical consequences of any particular hypothesis in regard to them.

### CORRELATION

Relations between variables which can be measured quantitatively are usually expressed in terms of Galton's (*4*)[1] coefficient of correlation, $r_{XY}=\frac{\Sigma X'Y'}{n\sigma_X\sigma_Y}$ (the ratio of the average product of deviations of $X$ and $Y$ to the product of their standard deviations), or of Pearson's (*7*) correlation ratio, $\eta_{X\cdot Y}=\frac{\sigma\left(\frac{M}{Y}_X\right)}{\sigma_X}$ (the ratio of the standard deviation of the mean values of $X$ for each value of $Y$ to the total standard deviation of $X$), the standard deviation being the square root of the mean square deviation.

Use of the coefficient of correlation ($r$) assumes that there is a linear relation between the two variables—that is, that a given change in one variable always involves a certain constant change in the corresponding average value of the other. The value of the coefficient can never exceed

[1] Reference is made by number (italic) to "Literature cited," p. 585.

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+1 or −1. For many purposes it is enough to look on it as giving an arbitrary scale between +1 for perfect positive correlation, 0 for no correlation, and −1 for perfect negative correlation.

The correlation ratio ($\eta$) equals the coefficient of correlation if the relation between the variables is exactly linear. It does not, however, depend on the assumption of such a relation, and it is always larger than $r$ when the relations are not exactly linear. It can only take values between 0 and +1, and it can be looked upon as giving an arbitrary scale between 0 for no correlation and 1 for perfect correlation.

The numerical value of the coefficient of correlation ($r$) takes on added significance in connection with the idea of regression. It gives the average deviation of either variable from its mean value corresponding to a given deviation of the other variable, provided that the standard deviation is the unit of measurement in both cases. The regression in terms of the actual units can, of course, be obtained by multiplying by the ratio of the standard deviations. Thus, for the deviation of $X$ corresponding to a unit deviation of $Y$, we have $reg_{X \cdot Y} = r_{XY}\frac{\sigma_X}{\sigma_Y}$. This formula may be deduced from the theory of least squares as the best linear expression for $X$ in terms of $Y$. The formula for what Galton later called the coefficient of correlation was, in fact, first presented in this connection by Bravais (1) in 1846. Any such interpretation is of course impossible with the correlation ratio.

The numerical values of both coefficients, however, have significance in another way. Their squares ($\eta^2$, or $r^2$ if regression is linear) measure the portion of the variability of one of the variables which is determined by the other and which disappears in data in which the second is constant. Thus if ${}_Y\sigma^2_X$ is the mean square deviation of $X$ for constant $Y$, Pearson has shown that:

$$ {}_Y\sigma^2_X = \sigma^2_X(1 - \eta^2_{X \cdot Y}) $$

or ${}_Y\sigma^2_X = \sigma^2_X(1 - r^2_{XY})$ if regression is linear.

It often happens that it is desirable to consider simultaneously the relations in a system of more than two variables. For such cases, involving only linear relations between the various pairs of variables, Pearson (6) has devised the coefficient of multiple correlation.

$$ R_{X(ABC\cdots N)} = \sqrt{1 - \frac{\Delta}{\Delta_{XX}}} $$

in which

$$ \Delta = \begin{vmatrix} 1 & r_{XA} & r_{XB} & \cdot & \cdot & r_{XN} \\ r_{AX} & 1 & r_{AB} & \cdot & \cdot & r_{AN} \\ r_{BX} & r_{BA} & 1 & \cdot & \cdot & r_{BN} \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ r_{NX} & r_{NA} & r_{NB} & \cdot & \cdot & 1 \end{vmatrix} $$

and $\Delta_{XX}$ is the minor made by deleting row $X$ and column $X$. $R^2_{X(ABC\cdots N)}$ measures the degree of determination of $X$ by the whole set of other factors, and $1-R^2_{X(ABC\cdots N)}=\frac{\Delta}{\Delta_{XX}}$ is the maximum possible squared correlation between $X$ and a factor independent of those considered. This formula for multiple correlation leads to one for multiple regression. Letting $X'$, $A'$, $B'$, etc., be the deviations of variables $X$, $A$, $B$, etc., from their mean values, Pearson has shown that the most probable value of $X'$ for known values of the other variables is given by the formula

$$\frac{X'}{\sigma_X}=\frac{\Delta_{XA}}{\Delta_{XX}}\frac{A'}{\sigma_A}+\frac{\Delta_{XB}}{\Delta_{XX}}\frac{B'}{\sigma_B}\quad\cdots\cdots\quad\frac{\Delta_{XN}}{\Delta_{XX}}\frac{N'}{\sigma_N}$$

$$\sigma_{X'}=_{N\cdots BA}\sigma_X=\sigma_X\sqrt{\frac{\Delta}{\Delta_{XX}}}.$$

Analogous but more complex formulae have recently been published by Isserlis (5) for the multiple correlation ratio for use in cases in which the regressions are not necessarily linear.

## CAUSATION

In all the preceding results no account is taken of the nature of the relationship between the variables. The calculations thus neglect a very important part of the knowledge which we often possess. There are usually *a priori* or experimental grounds for believing that certain factors are direct causes of variation in others or that other pairs are related as effects of a common cause. In many cases, again, there is an obvious mathematical relationship between variables, as between a sum and its components or between a product and its factors. A correlation between the length and volume of a body is an example of this kind. Just because it involves no assumptions in regard to the nature of the relationship, a coefficient of correlation may be looked upon as a fact pertaining to the description of a particular population only to be questioned on the grounds of inaccuracy in computation. But it would often be desirable to use a method of analysis by which the knowledge that we have in regard to causal relations may be combined with the knowledge of the degree of relationship furnished by the coefficients of correlation.

The problem can best be presented by using a concrete example. In a population of guinea pigs it will be found that the birth weights, early gains, sizes of litters, and gestation periods are all more or less closely correlated with each other. The influence of heredity, environmental conditions, health of dam, etc., are also easily shown. In a rough way, at least, it is easy to see why these variables are correlated with each other. These relations can be represented conveniently in a diagram like that in figure 1, in which the paths of influence are shown by arrows.

The variety and complexity of the relations which may be back of a correlation are well illustrated in this case. Thus, the weight at weaning (33 days of age) should be correlated with the birth weight and with the gain between birth and weaning simply because it is their sum. The relations of birth weight with gestation period and the prenatal rate of growth are also essentially mathematical rather than causal. Birth weight is necessarily fully determined by the character of the prenatal growth curve and the time at which this is interrupted by birth.

In the relation between gestation period and size of litter we come to a case in which there is no necessary mathematical relationship. We naturally attempt to account for the high negative correlation by the hypothesis that a large number in a litter in some way causes early

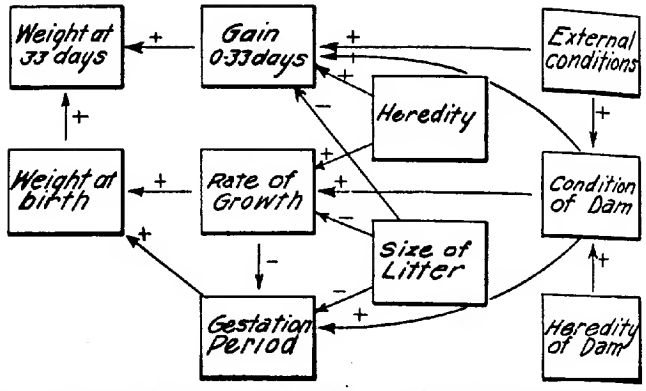


FIG. 1.—Diagram illustrating the interrelations among the factors which determine the weight of guinea pigs at birth and at weaning (33 days).

parturition. Similarly, a large number in a litter might be expected to be a cause of slow growth in the foetuses.

Birth weight and gain after birth are highly correlated. Here neither variable can be spoken of as the cause of variation in the other, and the relation is not mathematical. They are evidently influenced by common causes, among which heredity, size of litter, and conditions which affect the health of the dam up to the time of birth at once come to mind.

Most of the variables are connected with each other through more than one path. Thus, weight at birth is correlated with weight at weaning both as a component of a sum and as the effect of common causes.

There may be a conflict of the paths. Thus, a large number in a litter has a fairly direct tendency to shorten the gestation period, but this is probably balanced in part by its tendency to reduce the rate of growth of the foetuses, slow growth permitting a longer gestation period. Large litters tend to reduce gestation period and rate of growth before and after birth. But large litters are themselves most apt to come when

external conditions are favorable, which also favors long gestation periods and vigorous growth.

The coefficient of correlation is a resultant of all paths connecting the two variables. It would be valuable in many cases to be able to determine the relative importance of each particular path. The usual method in such cases is to calculate the partial correlation between two variables for a third constant, using Pearson's well-known formula

$$_{C}r_{AB}=\frac{r_{AB}-r_{AC}r_{BC}}{\sqrt{(1-r^2_{AC})(1-r^2_{BC})}}$$

for correlation between $A$ and $B$ for constant $C$. Such partial correlations, however, must be interpreted with caution. It is true that by making constant a connecting link between two variables, whether it is a common cause or the cause of one and effect of the other, we eliminate the path in question. This elimination of connecting paths in which the constant factor is a link is not, however, the only way in which correlation is affected. If an effect of a number of causes is made constant, spurious negative correlations appear among the causes and their other effects. Thus, if weight at 33 days is made constant, the correlation between birth weight and gain necessarily becomes $-1$. We are simply picking out a population in which any deficiencies in birth weight happen to be exactly balanced by excess in gain after birth. This is an extreme case, but where the relations of cause and effect are at all complex it is evident that the correlation between two variables may be changed in more than one way by making a third variable constant, making the interpretation doubtful.

Where there is a network of causes and effects, the interrelations could be grasped best if a coefficient could be assigned to each path in the diagram designed to measure the direct influence along it. The following is an attempt to provide such a coefficient, which may be called a path coefficient.

DEFINITIONS

We will start with the assumption that the direct influence along a given path can be measured by the standard deviation remaining in the effect after all other possible paths of influence are eliminated, while variation of the causes back of the given path is kept as great as ever, regardless of their relations to the other variables which have been made constant. Let $X$ be the dependent variable or effect and $A$ the independent variable or cause. The expression $\sigma_{X\cdot A}$ will be used for the standard deviation of $X$, which is found under the foregoing conditions, and may be read as the standard deviation of $X$ due to $A$. In a system in which variation of $X$ is completely determined by $A$, $B$, and $C$ we have $\sigma_{X\cdot A}={}_{A}r_{CB}\sigma_{X}$ representing the constant factors, $B$ and $C$, and also the variation of $A$ itself ($\sigma_{A}$) by subscripts to the left. The path

coefficient for the path from $A$ to $X$ will be defined as the ratio of the standard deviation of $X$ due to $A$ to the total standard deviation of $X$.

$$p_{X\cdot A}=\frac{\sigma_{X\cdot A}}{\sigma_X}.$$

Just as the regression of $X$ on $A$ is expressed by $r_{XA}\frac{\sigma_X}{\sigma_A}$ the deviation of $X$ directly caused by a unit deviation of $A$ is given by the formula

$$p_{X\cdot A}\frac{\sigma_X}{\sigma_A}=\frac{\sigma_{X\cdot A}}{\sigma_A}.$$

Another coefficient which it will be convenient to use, the coefficient of determination of $X$ by $A$, $d_{X\cdot A}$, measures the fraction of complete determination for which factor $A$ is directly responsible in the given system of factors. This definition implies that the sum of such coefficients must equal unity if all causes are accounted for.

## SYSTEMS OF INDEPENDENT CAUSES

The degree of determination of one variable by another is most easily found where the variables are connected by a mathematical relationship. The simplest mathematical relationship is that between a sum and its components. For the standard deviation of a sum the following relation is well known:

$$\sigma^2_{A+B}=\frac{\Sigma(A'+B')^2}{n}=\sigma^2_A+\sigma^2_B+2\sigma_A\sigma_B r_{AB}.$$

If $A$ and $B$ are independent of each other, $r_{AB}=0$, and we have

$$\sigma^2_{A+B}=\sigma^2_A+\sigma^2_B.$$

The degree to which variation of the sum is determined by that of each component is obvious.

$$d_{X\cdot A}=\frac{\sigma^2_A}{\sigma^2_X} \text{ and } d_{X\cdot B}=\frac{\sigma^2_B}{\sigma^2_X}, \text{ where } X=A+B,$$

giving $d_{X\cdot A}+d_{X\cdot B}=1$, as required by definition.

For the standard deviation of $X$ due to $A$ we have in this case, $\sigma_{X\cdot A}=\sigma_A$. Thus, $p_{X\cdot A}=\frac{\sigma_{X\cdot A}}{\sigma_X}=\frac{\sigma_A}{\sigma_X}$ by definition.

Again, $r_{XA}=\frac{\Sigma(A'+B')A'}{n\sigma_X\sigma_A}=\frac{\Sigma A'^2}{n\sigma_X\sigma_A}=\frac{\sigma_A}{\sigma_X}$.

Summing up, $p_{X\cdot A}=\sqrt{d_{X\cdot A}}=r_{XA}$.

It can easily be shown that the same formulae hold in case we are dealing with the sum of multiples of a number of independent factors instead of with their own sum.

We can pass at once from this case to cases in which variation of $X$ is caused in the physical or physiological sense by variation in several causes

provided that these causes are independent of each other, have linear relations to the dependent variable $X$, and that the deviations which they determine are additive. They are independent of each other if there is no correlation between their variations. A cause has a linear relation to the effect and is combined additively with the other factors if a given amount of change in it always determines the same change in the effect, regardless of its own absolute value or that of the other causes. The conclusion is that, under these conditions, the path coefficient equals the coefficient of correlation between cause and effect, and the degree of determination equals the square of either of the preceding coefficients.

CHAINS OF CAUSES

If we know the extent to which a variable $X$ is determined by a certain cause $M$, which is independent of other causes, combines with them additively, and acts on $X$ in a linear manner, and if we know the extent to which $M$ is determined by a more remote cause $A$, the degree of determination of $X$ by $A$ must be the product of the component degrees of determination.

Let $X = M + N$, and $M = A + B$

$$d_{X \cdot M} = \frac{\sigma^2_M}{\sigma^2_X},\ d_{M \cdot A} = \frac{\sigma^2_A}{\sigma^2_M},\ \text{and}\ d_{X \cdot A} = \frac{\sigma^2_A}{\sigma^2_X}.$$

Thus $d_{X \cdot A} = d_{X \cdot M} d_{M \cdot A}$

and $p_{X \cdot A} = p_{X \cdot M} p_{M \cdot A}$.

NONADDITIVE FACTORS

In cases in which a factor does not act additively with the other factors in determining the variations in the dependent variable, its influence on the latter can not be completely expressed apart from the other factors, at least in terms of the ordinary measures of variability. This can be made clearer by an illustration. Multiplying factors are among the most important of those which do not combine by addition.

Let $X = AB$ and assume that $r_{AB} = o$

$$\sigma^2_X = M^2_B \sigma^2_A + M^2_A \sigma^2_B + \frac{\Sigma A'^2 B'^2}{n}$$

where $A'$ and $B'$ are deviations of $A$ and $B$ from their mean values $M_A$ and $M_B$. Putting $B$ constant, we have $\sigma^2_{X \cdot A} = M^2_B \sigma^2_A$; and similarly putting $A$ constant, we have $\sigma^2_{X \cdot B} = M^2_A \sigma^2_B$. There remains a portion of $\sigma^2_X$ which is due to $A$ and $B$ jointly and which can not be separated into parts due to each alone. If we write $d_{X \cdot A} = \frac{M^2_B \sigma^2_A}{\sigma^2_X}$ as the degree of determination of $X$ by variation of $A$ alone, and $d_{X \cdot B} = \frac{M^2_A \sigma^2_B}{\sigma^2_X}$ as the corresponding degree of determination of $X$ by variation of $B$ alone, we must recognize an additional term $d_{X \cdot \overline{AB}} = \frac{\Sigma A'^2 B'^2}{n \sigma^2_X}$ in order that the sum of the

coefficients of determination may equal unity. Regression is linear and $r^2_{XA}=\eta^2_{X\cdot A}=\frac{M^2_B\sigma^2_A}{\sigma^2_X}$. Thus $d_{X\cdot A}=r^2_{XA}$ as in the case of independent additive factors. The term $\frac{\Sigma A'^2B'^2}{n\sigma^2_X}$ is small unless the amounts of variation in $A$ and $B$ are large in comparison with the mean values. In many cases it is safe to deal with path coefficients and degrees of determination in the case of multiplying factors just as in the case of additive factors.

As a concrete illustration of these points take two independent variables, for each of which the values 1, 2, and 3 occur in the frequencies 1, 2, and 1, respectively. Below is the correlation table between one of these factors and their product.

Product (X).

| Factor (A). | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1..... | 1 | 2 | 1 | .... | .... | .... | .... | .... | .... | 4 |
| 2..... | .... | 2 | .... | 4 | .... | 2 | .... | .... | .... | 8 |
| 3..... | .... | .... | 1 | .... | .... | 2 | .... | .... | 1 | 4 |
| | 1 | 4 | 2 | 4 | 0 | 4 | 0 | 0 | 1 | 16 |

$M_A=2 \quad \sigma_A=\sqrt{1/2} \quad r_{AX}=\sqrt{8/17} \quad d_{X\cdot A}=8/17$

$M_X=4 \quad \sigma_X=\sqrt{17/4} \quad \frac{\Sigma A'^2B'^2}{n\sigma^2_X}=1/17 \quad d_{X\cdot B}=8/17$

$d_{X\cdot\overline{AB}}=\frac{1/17}{1}$

In this case the amounts of variation in the factors are relatively large compared with their mean values, making the distribution surface markedly heteroscedastic, yet the degree of determination by either factor comes out only slightly less than one-half.

## NONLINEAR RELATIONS

Pearson's definition of the correlation ratio, $\eta_{X\cdot A}=\frac{\sigma({}_A{}^M{}_X)}{\sigma_X}$, has already been given. The variations of the mean value of $X$ for different values of $A$ are the variations which can be attributed to the direct influence of $A$, assuming that $A$ is cause, $X$ effect, and that other causes are combined with $A$ additively. Thus $\sigma_{X\cdot A}=\sigma({}_A{}^M{}_X)$ and we have at once $p_{X\cdot A}=\eta_{X\cdot A}$.

Again, as the total variation of $X$ is composed of the variation of its mean values for different values of $A$, plus the variation about these mean values, we have $\sigma^2_X=\sigma^2({}_A{}^M{}_X)+{}_A\sigma^2_X$, giving ${}_A\sigma^2_X=\sigma^2_X\,(1-\eta^2_{X\cdot A})$, as already noted.

Thus $\eta^2_{X\cdot A}$ measures the portion of $\sigma^2_X$ lost by making $A$ constant, so that as before $d_{X\cdot A}=\eta^2_{X\cdot A}=p^2_{X\cdot A}$.

Unfortunately we can not deal with chains of factors which involve nonlinear relations by mere multiplication of the path coefficients of the component links. In the present paper, unless otherwise stated, it will be assumed that all correlations are essentially linear.

### EFFECTS OF COMMON CAUSES

Suppose that two variables, $X$ and $Y$, are affected by a number of causes in common, ($B$, $C$, $D$). Let $A$ represent causes affecting $X$ alone and $E$ causes affecting $Y$ alone (fig. 2).

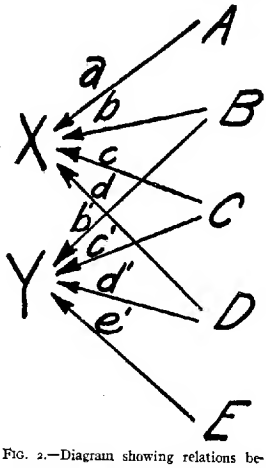


FIG. 2.—Diagram showing relations between two variables, $X$ and $Y$, whose values are determined in part by common causes, $B$, $C$, and $D$, which are independent of each other.

Let $p_{X\cdot A}=a$ $p_{Y\cdot A}=o$
$p_{X\cdot B}=b$ $p_{Y\cdot B}=b'$
$p_{X\cdot C}=c$ $p_{Y\cdot C}=c'$
$p_{X\cdot D}=d$ $p_{Y\cdot D}=d'$
$p_{X\cdot E}=o$ $p_{Y\cdot E}=e'$

$B$, $C$, and $D$ are assumed to be independent of each other—that is, $r_{BC}=0$, etc.

Hence $p_{X\cdot B}=r_{XB}$, etc.

$$_{B}r_{XY}=\frac{r_{XY}-bb'}{\sqrt{(1-b^2)\,(1-b'^2)}}$$

$$_{CB}r_{XY}=\frac{_{B}r_{XY}-{}_{B}r_{XC}\;{}_{B}r_{YC}}{\sqrt{(1-{}_{B}r^2_{XC})\,(1-{}_{B}r^2_{YC})}}=\frac{r_{XY}-bb'-cc'}{\sqrt{(1-b^2-c^2)(1-b'^2-c'^2)}}.$$

When all common causes have been made constant, $_{DCB}r_{XY}=0$

$$r_{XY}=bb'+cc'+dd'=\Sigma p_{X\cdot B}p_{Y\cdot B}.$$

Thus, in those cases in which the causes are independent of each other, the correlation between two variables equals the sum of the products of the pairs of path coefficients which connect the two variables with each common cause. An illustration of the use of this principle was given in an earlier paper (*8*) in analyzing the nature of size factors in rabbits.

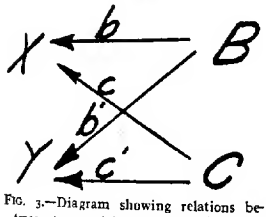


FIG. 3.—Diagram showing relations between two variables, $X$ and $Y$, whose values are completely determined by common causes, $B$ and $C$, which are independent of each other.

It may be deduced from the foregoing formula that two variables may even be completely determined by the same factors and yet be uncorrelated with each other. Let variation of $X$ be completely determined by factors $B$ and $C$, the path coefficients being $b$ and $c$, respectively. Let $Y$ be completely determined by the same factors, the path coefficients being $b'$ and $c'$ (fig. 3). Then $r_{XY}=bb'+cc'$. The condition

under which $r_{XY}$ may equal zero is evidently that $bb' = -cc'$. An example may be found in the absence of correlation between the sum and difference of pairs of numbers picked at random from a table.

In many cases a small actual correlation between variables will be found on analysis to be the resultant of a balancing of very much more important but opposed paths of influence leading from common causes.

## SYSTEMS OF CORRELATED CAUSES

The discussion up to this point has dealt wholly with causes which act independently of each other. It is necessary to consider the effects of correlation among the causes.

Let us consider the sum of two correlated variables (fig. 4).

$$\text{Let } X = M + N$$
$$\sigma^2_X = \sigma^2_M + \sigma^2_N + 2\sigma_M\sigma_N r_{MN}.$$

We have defined $\sigma_{X \cdot M}$ as the standard deviation of $X$ when factors other than $M$ are constant, but $M$ varies as much as before. The latter qualification is important in the present case, since the making of $N$ constant tends to reduce the variation of $M$, reducing $\sigma_M$ to $\sigma_M\sqrt{1-r^2_{MN}}$. The definition of $\sigma_{X \cdot M}$ implies that not only is $N$ made constant but that there is such a readjustment among the more remote causes, $A$, $B$, and $C$, that $\sigma_M$ is unchanged. Under the definition it is evident that in this case $\sigma_{X \cdot M} = \sigma_M$ and $\sigma_{X \cdot N} = \sigma_N$. Thus $p_{X \cdot M} = \frac{\sigma_M}{\sigma_X}$ and $p_{X \cdot N} = \frac{\sigma_N}{\sigma_X}$.

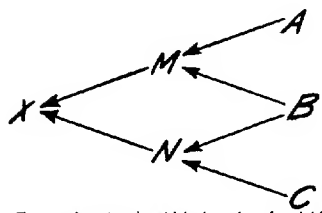


FIG. 4.—A system in which the value of variable $X$ is completely determined by causes $M$ and $N$, which are correlated with each other.

In attempting to find the degrees of determination of $X$ by $M$ and $N$ we meet a difficulty somewhat similar to that met in the case of non-additive factors. The squared standard deviation is made up in part of elements due wholly to $M$ and $N$, respectively, but in part to a portion which can not be divided between them. The term $2\sigma_M\sigma_N r_{MN}$ is due solely to the fact that the variations of $X$, which $M$ and $N$ determine, tend to be in the same direction and so have greater effect than if variations $M$ and $N$ were combined at random. It seems best to define $d_{X \cdot M}$ as the degree of determination of $X$ due to $M$ alone. Thus $d_{X \cdot M} = \frac{\sigma^2_M}{\sigma^2_X}$, $d_{X \cdot N} = \frac{\sigma^2_N}{\sigma^2_X}$. The remaining term may be considered as determination by $M$ and $N$ jointly and may be written $d_{X \cdot \overline{MN}} = 2p_{X \cdot M}p_{X \cdot N}r_{MN}$.

These rules can be extended at once to the sums of more than two variables, to sums of multiples of variables, and hence, as before, to

linear relations of cause and effect in which the influence of the causes is combined additively. It is also easy to show that the formulae apply approximately for multiplying factors.

Summing up, $p_{X\cdot M}=\sqrt{d_{X\cdot M}}=\frac{\sigma_{X\cdot M}}{\sigma_X}$

$$\Sigma d_{X\cdot M}+2\Sigma p_{X\cdot M}p_{X\cdot N}r_{MN}=1.$$

The next problem is to find the degree of determination of $X$ by a factor such as $B$, which is connected with $X$ by more than one path (fig. 5).

Assume that $A$, $B$, $C$, and $D$ are independent and completely determine $X$. $d_{X\cdot A}+d_{X\cdot B}+d_{X\cdot C}+d_{X\cdot D}=1$. But also $d_{X\cdot M}+d_{X\cdot N}+2p_{X\cdot M}p_{X\cdot N}r_{MN}+d_{X\cdot D}=1$.

$d_{X\cdot B}=d_{X\cdot M}-d_{X\cdot A}+d_{X\cdot N}-d_{X\cdot C}+2p_{X\cdot M}p_{X\cdot N}p_{M\cdot B}p_{N\cdot B}$, remembering that $r_{MN}=p_{M\cdot B}p_{N\cdot B}$.

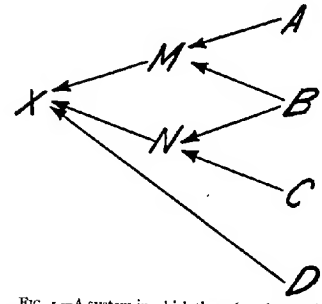


FIG. 5.—A system in which the value of $X$ is affected by a factor, $B$, along two different paths, $BMX$ and $BNX$.

Since $d_{M\cdot A}+d_{M\cdot B}=1$, etc., we have $d_{X\cdot M}=d_{X\cdot M}d_{M\cdot A}+d_{X\cdot M}d_{M\cdot B}=d_{X\cdot A}+d_{X\cdot M}d_{M\cdot B}$, and $d_{X\cdot N}=d_{X\cdot C}+d_{X\cdot N}d_{N\cdot B}$.

Therefore $d_{X\cdot B}=d_{X\cdot M}d_{M\cdot B}+d_{X\cdot N}d_{N\cdot B}+2p_{X\cdot M}p_{X\cdot N}p_{M\cdot B}p_{N\cdot B}$

$$=p^2_{X\cdot M}p^2_{M\cdot B}+p^2_{X\cdot N}p^2_{N\cdot B}+2p_{X\cdot M}p_{X\cdot N}p_{M\cdot B}p_{N\cdot B}$$

$$=(p_{X\cdot M}p_{M\cdot B}+p_{X\cdot N}p_{N\cdot B})^2$$

$$p_{X\cdot B}=p_{X\cdot M}p_{M\cdot B}+p_{X\cdot N}p_{N\cdot B}.$$

These results are easily extended to cases in which $B$ acts on $X$ through any number of causes. If a path coefficient is assigned to each component path, the combined path coefficient for all paths connecting an effect with a remote cause equals the sum of the products of the path coefficients along all the paths. Since $B$ is independent of $A$, $C$, and $D$, $r_{X\cdot B}=p_{X\cdot B}=p_{X\cdot M}p_{M\cdot B}+p_{X\cdot N}p_{N\cdot B}$.

## GENERAL FORMULA

We are now in a position to express the correlation between any two variables in terms of path coefficients. Let $X$ and $Y$ be two variables which are affected by correlated causes $M$ and $N$. Represent the various path coefficients by small letters as in the diagram. Let $A$, $B$, and $C$ be hypothetical remote causes which are independent of each other (fig. 6).

$$r_{XY}=p_{X\cdot A}p_{Y\cdot A}+p_{X\cdot B}p_{Y\cdot B}+p_{\cdot C}p_{Y\cdot C}$$

$$=mam'a+(mb+nb')(m'b+n'b')+ncn'c$$

$$=mm'+mbb'n'+nn'+nb'bm'.$$

Thus, the correlation between two variables is equal to the sum of the products of the chains of path coefficients along all of the paths by which they are connected.

If we know only the effects, $X$ and $Y$, and correlated causes, such as $M$ and $N$, it will be well to substitute $r_{MN}$ for $bb'$ in the foregoing formula.

$$r_{XY} = p_{X\cdot M}p_{Y\cdot M} + p_{X\cdot M}r_{MN}p_{Y\cdot N} + p_{X\cdot N}p_{Y\cdot N} + p_{X\cdot N}r_{MN}p_{Y\cdot M}.$$

We have reached a general formula expressing correlation in terms of path coefficients. This is not the order in which knowledge of the coefficients must be obtained, but, nevertheless, by means of simultaneous equations the values of the path coefficients in a system can often be calculated from the known correlations. Additional equations are furnished by the principle that the sum of the degrees of determination must

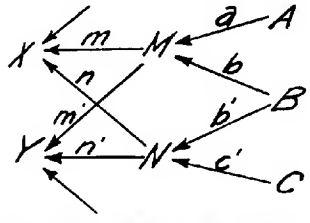


FIG. 6. Diagram showing relations between two variables, $X$ and $Y$, whose values are determined in part by common causes, $M$ and $N$, which are correlated with each other.

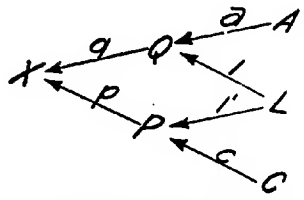


FIG. 7.—Simplified diagram of factors which determine birth weight in guinea pigs.

equal unity. The fundamental equations can be written in general form as follows:

$$d_{X\cdot A} = p^2_{X\cdot A}$$

$$d_{X\cdot\overline{AB}} = 2p_{X\cdot A}p_{X\cdot B}r_{AB}$$

$$\Sigma d_{X\cdot A} + \Sigma d_{X\cdot\overline{AB}} = 1$$

$$r_{XY} = \Sigma p_{X\cdot A}p_{Y\cdot A}.$$

## APPLICATION TO BIRTH WEIGHT OF GUINEA PIGS

As a simple example, we may consider the factors which determine birth weight in guinea pigs (fig. 7).

Let $X$ be birth weight; $Q$, prenatal growth curve; $P$, gestation period; $L$, size of litter; $A$, hereditary and environmental factors which determine $Q$, apart from size of litter; $C$, factors determining gestation period apart from size of litter.

For the sake of simplicity, it will be assumed that the interval between litters (if less than 75 days) accurately measures the gestation period

and that the variables are connected only by the paths shown above. In a certain stock of guinea pigs the following correlations were found:

Birth weight with interval, $r_{XP} = +0.5547$.

Birth weight with litter, $r_{XL} = -0.6578$.

Interval with litter, $r_{PL} = -0.4444$.

We are able to form three equations of type $r_{XY} = \Sigma p_{X \cdot A} p_{Y \cdot A}$ and three of type $\Sigma p^2_{X \cdot A} + 2\Sigma p_{X \cdot A} p_{X \cdot B} r_{AB} = 1$. These six equations will enable us to calculate six unknown quantities. The six path coefficients in the diagram in figure 7 can thus be calculated from the information given here, but no others.

The equations are as follows:

(1) $r_{XP} = +0.5547 = p + qll'$.

(2) $r_{XL} = -0.6578 = ql + pl'$.

(3) $r_{PL} = -0.4444 = l'$.

(4) $q^2 + p^2 + 2qpll' = 1$.

(5) $a^2 + l^2 = 1$.

(6) $l'^2 + c^2 = 1$.

| | | | | |
|---|---|---|---|---|
| From (3), | $p_{P \cdot L} = l' = -0.4444$ | | $d_{P \cdot L} = l'^2$ | $= 0.1975$ |
| From (6), | $p_{P \cdot C} = c = 0.8958$ | | $d_{P \cdot C} = c^2$ | $= .8025$ |
| | | | | $1.0000$ |
| From (1) and (2), | $p_{X \cdot P} = p = 0.3269$ | | $d_{X \cdot P} = p^2$ | $= 0.1069$ |
| | $ql = -0.5125$ | | $d_{X \cdot Q} = q^2$ | $= .7442$ |
| From (4), | $p_{X \cdot Q} = q = 0.8627$ | | $d_{X \cdot \overline{PQ}} = 2pqll'$ | $= .1489$ |
| | | | | $1.0000$ |
| | $p_{Q \cdot L} = l = -0.5941$ | | $d_{Q \cdot L} = l^2$ | $= 0.3530$ |
| | $p_{Q \cdot A} = a = 0.8044$ | | $d_{Q \cdot A} = a^2$ | $= .6470$ |
| | | | | $1.0000$ |
| | | | $d_{X \cdot Q \cdot L} = q^2 l^2$ | $= 0.2627$ |
| | | | $d_{X \cdot P \cdot L} = p^2 l'^2$ | $= .0211$ |
| | | | $d_{X \cdot \overline{PQ} \cdot L} = 2pqll'$ | $= .1489$ |
| | | | $d_{X \cdot L} = (ql + pl')^2$ | $= .4327$ |
| | | | $d_{X \cdot A} = q^2 a^2$ | $= .4815$ |
| | | | $d_{X \cdot C} = p^2 c^2$ | $= .0858$ |
| | | | | $1.0000$ |

Assuming that the diagrams in figures 7, 8, and 9 accurately represent the causal relations, it appears that birth weight is determined to a very much greater extent by variations in the rate of growth of the foetuses than by variations in the length of the gestation period ($d_{X \cdot Q}=0.74$, $d_{X \cdot P}=0.11$). Size of litter has much more effect on birth weight by reducing the rate of growth of the foetuses than by causing early parturition ($d_{X,Q,L}=0.26$, $d_{X,P,L}=0.02$). The difference in birth weight caused by a difference of a day in gestation period can be calculated from the path coefficient and the standard deviations by the formula for path regression, *p. reg*$_{X \cdot P}=p_{X \cdot P}\frac{\sigma_X}{\sigma_P}$. The result, 3.34 gm. per day, should measure the average rate of growth just preceding parturition. The actual regression, 5.66 gm. per day of delay in parturition, is larger because a long gestation period means not merely a longer time for growth but also, in general, a smaller litter and hence more rapid growth.

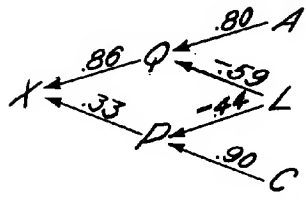


FIG. 8.—Path coefficients measuring the relations between birth rate ($X$), rate of growth ($Q$), gestation period ($P$), size of litter ($L$), and other causes ($A$, $C$).

On introducing other data the analysis can be carried much farther. There are other paths of influence which should be recognized, positive paths connecting $A$, $C$, and $L$, representing the favorable effects of good health in the dam on rate of growth, gestation period, and size of litter, and a negative path from $Q$ to $P$ to represent the tendency of rapid growth to induce early parturition. The relations between the observed interval between litters and the actual gestation period should also be considered. The results presented here are thus intended merely to furnish a simple illustration of the method. A more complete analysis of the relations among the factors which affect birth weight and later growth will be presented in a later paper.

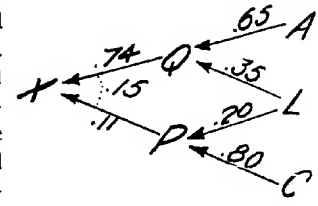


FIG. 9.—Coefficients of determination. Symbols as in figure 7.

## DETERMINATION IN TERMS OF CORRELATION

Having obtained a formula for correlation in terms of determination, the question arises whether the converse is possible. For a special class of cases such a formula is easily obtained.

For a single cause and effect the required formula is merely $d_{X \cdot A} = r^2_{XA}$ (fig. 10).

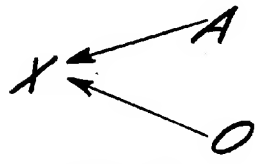


FIG. 10.—Effect and one known cause.

The degree of determination by residual factors; that is, $d_{X \cdot O}$, is thus $1 - r^2_{XA}$.

If two causes are known, and the degree of correlation between them, we have (fig. 11)—

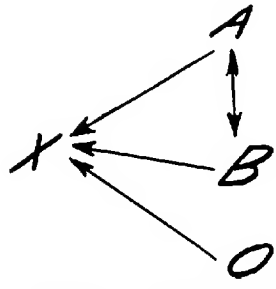


FIG. 11.—Effect and two correlated known causes.

$$ {}_B r^2_{XA} + {}_B r^2_{XO} = 1 $$

$$ \frac{(r_{XA} - r_{XB} r_{AB})^2}{(1 - r^2_{XB})(1 - r^2_{AB})} = 1 - \frac{r^2_{XO}}{1 - r^2_{XB}} $$

$$ r^2_{XO} = d_{X \cdot O} = \frac{1 - r^2_{XA} - r^2_{XB} - r^2_{AB} + 2 r_{XA} r_{XB} r_{AB}}{1 - r^2_{AB}}. $$

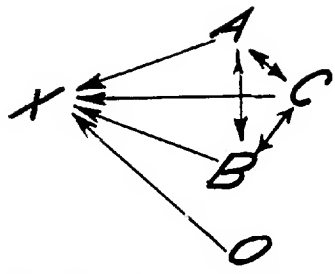


FIG. 12.—Effect and three correlated known causes.

If three causes and their correlations are known (fig. 12), we have ${}_{CB} r^2_{XA} + {}_{CB} r^2_{XO} = 1$, from which

$$ r^2_{XO} = d_{X \cdot O} = \frac{1 - \Sigma r^2_{XA} + 2\Sigma r_{XA} r_{AB} r_{BX} - 2\Sigma r_{XA} r_{AB} r_{BC} r_{CX} + \Sigma r^2_{XA} r^2_{BC}}{1 - r^2_{AB} - r^2_{AC} - r^2_{BC} + 2 r_{AC} r_{CB} r_{BA}}. $$

In this expression $\Sigma r^2_{XA}$ means the sum of squares of the six known correlations. $\Sigma r_{XA}r_{AB}r_{BX}$ means the sum of the products of the groups of three correlations, corresponding to the sides of triangles. There are four of these triangles, *XAC*, *XAB*, *XCB*, *ABC*. $\Sigma r_{XA}r_{AB}r_{BC}r_{CX}$ means the sum of the three products of the groups of correlations which are arranged in closed quadrilaterals, and $\Sigma r^2_{XA}r^2_{BC}$ means the sum of the product of squared correlations in pairs which involve no common variable ($r^2_{XA}r^2_{BC}$, $r^2_{XC}r^2_{AB}$, $r^2_{XB}r^2_{AC}$) (fig. 13).

The formula for four known causes is easily found by a continuation of the methods used to find the others if attention is paid to the symmetry of the expressions. Since, however, this formula, as well as that just given for the case of three causes, is somewhat cumbersome, it will be convenient to use a more condensed notation. $\phi(XABC\ldots)$ may be used for a function involving all possible correlations among the variables $(XABC\ldots)$. In the definitions $\Sigma r^2$ means the sum of the squares of all correlations; $\Sigma r^2r^2$, the sum of the product of all pairs of squared correlations which involve no variables in common; $\Sigma rrr$, $\Sigma rrrr$, and $\Sigma rrrrr$ are the sums of the products of all groups of correlations which, represented by paths, form closed figures, triangles, quadrilaterals, and pentagons, respectively. $\Sigma r^2rrr$ is the sum of the products made by multiplying each triangle of correlations in the sense above by the second power of those correlations which do not involve any of the variables in the triangle. The number of terms of each kind is given above the brace, where it is more than one.

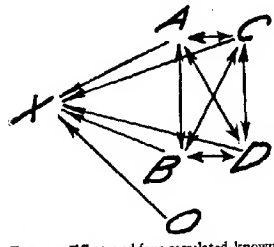


FIG. 13.—Effect and four correlated known causes.

$$\phi(AB) = 1 - r^2 \text{ (2 terms)}.$$

$$\phi(ABC) = 1 - \overbrace{\Sigma r^2}^{3} + 2\Sigma rrr \text{ (5 terms)}.$$

$$\phi(ABCD) = 1 - \overbrace{\Sigma r^2}^{6} + \overbrace{2\Sigma rrr}^{4} - \overbrace{2\Sigma rrrr}^{3} + \overbrace{\Sigma r^2r^2}^{3} \text{ (17 terms)}.$$

$$\phi(ABCDE) = 1 - \overbrace{\Sigma r^2}^{10} + \overbrace{2\Sigma rrr}^{10} - \overbrace{2\Sigma rrrr}^{15} + \overbrace{2\Sigma rrrrr}^{12} + \overbrace{\Sigma r^2r^2}^{15} - \overbrace{2\Sigma r^2rrr}^{10} \text{ (73 terms)}.$$

The formulae for degree of determination by residual factors may be written as follows:

$d_{X\cdot O}=\phi(XA)$ in system $XA$.

$d_{X\cdot O}=\dfrac{\phi(XAB)}{\phi(AB)}$ in system $XAB$.

$d_{X\cdot O}=\dfrac{\phi(XABC)}{\phi(ABC)}$ in system $XABC$.

$d_{X\cdot O}=\dfrac{\phi(XABCD)}{\phi(ABCD)}$ in system $XABCD$.

The degree of determination by the known causes is now easily calculated. When all causes of variation in $X$ are constant except $A$, variation of $X$ is measured by ${}_{O\cdots CB}\sigma_X$ and variation of $A$ is measured by ${}_{O\cdots CB}\sigma_A$, writing the constant-factors as subscripts to the left. Assuming that the relation between $A$ and $X$ is linear, the deviation of $X$ determined by a unit deviation of $A$ should be constant, whatever the amount of variation in $A$. Thus:

$$p_{X\cdot A}\frac{\sigma_X}{\sigma_A}=\frac{\sigma_{X\cdot A}}{\sigma_A}=\frac{{}_{O\cdots CB}\sigma_X}{{}_{O\cdots CB}\sigma_A}.$$

In the case of the residual factor $O$, assumed to be independent of the known factors $A$, $B$, $C$, etc., ${}_{\cdots CBA}\sigma_O=\sigma_O$,
and we have $\sigma_{X\cdot O}={}_{\cdots CBA}\sigma_X$

$$d_{X\cdot O}=\frac{\phi(XABC\ldots)}{\phi(ABC\ldots)}=\frac{\sigma^2_{X\cdot O}}{\sigma^2_X}=\frac{{}_{\cdots CBA}\sigma^2_X}{\sigma^2_X}.$$

Thus:

$${}_{\cdots CBA}\sigma^2_X=\frac{\phi(XABC\ldots)}{\phi(ABC\ldots)}\sigma^2_X.$$

This should be the general formula for the squared standard deviation with a number of constant factors.
Hence:

$$\frac{\sigma^2_{X\cdot A}}{\sigma^2_A}=\frac{\phi(XBC\ldots O)}{\phi(BC\ldots O)}\sigma^2_X\Big/\frac{\phi(ABC\ldots O)}{\phi(BC\ldots O)}\sigma^2_A$$

$$\sigma^2_{X\cdot A}=\frac{\phi(XBC\ldots O)}{\phi(ABC\ldots O)}\sigma^2_X$$

$$p_{X\cdot A}=\sqrt{\frac{\phi(XBC\ldots O)}{\phi(ABC\ldots O)}}$$

$$d_{X\cdot A}=\frac{\phi(XBC\ldots O)}{\phi(ABC\ldots O)}=\frac{\phi(XBC\ldots)-d_{X\cdot O}\phi(BC\ldots)}{\phi(ABC\ldots)}.$$

The general formula for partial correlation can easily be expressed in the present terminology.

$$_{DCB\cdot A}\sigma^2_X = {}_{DCB}\sigma^2_X(1 - {}_{DCB}r^2_{XA})$$

$${}_{DCB}r^2_{XA} = 1 - \frac{{}_{DCB\cdot A}\sigma^2_X}{{}_{DCB}\sigma^2_X} = 1 - \frac{\phi(XABCD)\phi(BCD)}{\phi(ABCD)\phi(XBCD)}.$$

In some cases it may be of interest to find the degree of determination when a number of factors not in the direct path between cause and effect are assumed constant.

$${}_{UTS}d_{X\cdot A} = \frac{{}_{UTS}\sigma^2_{X\cdot A}}{{}_{UTS}\sigma^2_X} = \frac{(_0\ldots{}_{UTS}\ldots{}_{CB}\sigma^2_X)(_{UTS}\sigma^2_A)}{(_0\ldots{}_{UTS}\ldots{}_{CB}\sigma^2_A)(_{UTS}\sigma^2_X)}$$

$$= \frac{\phi(XBC\ldots STU\ldots O)\phi(ASTU)}{\phi(ABC\ldots STU)\phi(XSTU)}.$$

## RELATION TO MULTIPLE CORRELATION

The expressions defined as $\phi(XABC\ldots)$, etc., suggest the expansion of determinants. It is in fact easy to show that $\phi(XABC\ldots N) = \Delta$.

Where
$$\Delta = \begin{vmatrix} 1 & r_{XA} & r_{XB} & \cdot & \cdot & r_{XN} \\ r_{AX} & 1 & r_{AB} & \cdot & \cdot & r_{AN} \\ r_{BX} & r_{BA} & 1 & \cdot & \cdot & r_{BN} \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ r_{NX} & r_{NA} & r_{NB} & \cdot & \cdot & 1 \end{vmatrix}$$

The formula for Pearson's coefficient of multiple correlation has already been given, $R_{X(ABCO)} = \sqrt{1 - \frac{\Delta}{\Delta_{XX}}}$ where $\Delta_{XX}$ is the minor made by deleting row $X$, column $X$.

Evidently in this class of cases the coefficient of determination degenerates into a function of the coefficient of multiple correlation. For the degree of determination by residual factors we have

$$d_{X\cdot O} = \frac{\phi(XABC\ldots)}{\phi(ABC\ldots)} = \frac{\Delta}{\Delta_{XX}} = 1 - R^2_{X(ABC\cdots)}$$

in agreement with Pearson's results.

For the degree of determination by a known factor we have

$$d_{X\cdot A} = \frac{\phi(XBC\ldots O)}{\phi(ABC\ldots O)} = \frac{\phi(XBC\ldots) - d_{X\cdot O}\phi(BC\ldots)}{\phi(ABC\ldots)} = \frac{\Delta_{AA}\Delta_{XX} - \Delta\Delta_{AAXX}}{\Delta^2_{XX}}$$

$$= \frac{\Delta^2_{XA}}{\Delta^2_{XX}}.$$

$$p_{X\cdot A} = \frac{\Delta_{XA}}{\Delta_{XX}}.$$

The last formula brings out the close relation between the path coefficients and multiple regression. As already noted, the most probable deviation of $X$ for known deviations of $A$, $B$, $C$, etc., is given by the formula

$$\frac{X'}{\sigma_X} = \frac{\Delta_{XA}A'}{\Delta_{XX}\sigma_A} + \frac{\Delta_{XB}B'}{\Delta_{XX}\sigma_B} \cdots = p_{X \cdot A}\frac{A'}{\sigma_A} + p_{X \cdot B}\frac{B'}{\sigma_B} \cdots$$

As already stated, Pearson's coefficients of multiple correlation and regression were not devised especially for the analysis of causal relations. The formula for multiple regression, for example, gives the most probable value of one of the variates for given values of the others regardless of causal relations. In cases in which all the correlations are known in a system including an effect and a number of causes the method can be used to find the path coefficients and the degrees of determination of the effect by each cause in the sense used in this paper. Such cases in which the direct methods can be used are, however, relatively uncommon. Where the system of paths of influence is at all complex, involving perhaps hypothetical factors, the causal relations can be analyzed only by the indirect method of expressing the known correlations in terms of the unknown path coefficients, making the sums of the degrees of determination unity and solving the simultaneous equations.

## PART II. APPLICATION TO THE TRANSPIRATION OF PLANTS

A large body of experimental data on the factors which affect the rate of transpiration in plants has been published by Briggs and Shantz (*2*). These data are well adapted for use in illustrating the methods of analyzing causal relations presented in part I of this paper.

The experiments which are used in this paper were conducted at Akron, Colo., in 1914. A variety of crop plants were grown in sealed pots. The total transpiration was measured each day. Among the environmental factors studied were the total solar radiation during the day, the wind velocity, the air temperature (in the shade), the rate of evaporation from a shallow tank, and the wet-bulb depression (sheltered from sun but not wind). The correlations between the daily transpiration of each kind of plant and the integrated values of the environmental factors were published by Briggs and Shantz. In order to avoid the effect of seasonal change in the plants, the logarithms of the ratios of the transpiration on succeeding days were correlated with similar figures for the various factors. The correlations between the various environmental factors for the 100 days from June 18 to September 25, 1914, have been calculated by the writer from the data presented by Briggs and Shantz. This period covers all the crop periods but is longer than most of them. None of the correlations appeared to depart much from linearity.

The daily averages, the standard deviations, and the correlations are given in Table I.

TABLE I.—*Daily averages, standard deviations, and correlations from experiments on transpiration in crop plants made by Briggs and Shantz at Akron, Colo., 1914*

| | Wind. | Radiation. | Temperature. | Wet-bulb depression. | Evaporation. |
|---|---|---|---|---|---|
| | | CORRELATIONS | | | |
| Wind | | −0.01 ±0.07 | −0.02 ±0.07 | 0.28 ±0.06 | 0.38 ±0.06 |
| Radiation | −0.01 ±0.07 | | .47 ± .05 | .48 ± .05 | .68 ± .04 |
| Temperature | − .02 ± .07 | .47 ± .05 | | .59 ± .05 | .56 ± .05 |
| Wet-bulb depression | .28 ± .06 | .48 ± .05 | .59 ± .05 | | .83 ± .02 |
| Evaporation | .38 ± .06 | .68 ± .04 | .56 ± .05 | .83 ± .02 | |
| Small grains[a] | .22 ± .04 | .65 ± .03 | .71 ± .02 | .88 ± .01 | .87 ± .02 |
| Rye | .19 ± .10 | .65 ± .06 | .73 ± .05 | .94 ± .01 | .91 ± .02 |
| Sorghum, millet[b] | .218± .041 | .570± .030 | .654± .026 | .788± .018 | .773± .021 |
| Sudan grass (in inclosure) | .52 ± .07 | .55 ± .06 | .84 ± .03 | .83 ± .03 | .93 ± .01 |
| Sudan grass (in open) | .32 ± .08 | .52 ± .07 | .81 ± .03 | .85 ± .03 | .82 ± .03 |
| Dent corn | .28 ± .08 | [c].52 ± .06 | .71 ± .04 | .81 ± .03 | .79 ± .03 |
| Algerian corn | .33 ± .09 | .62 ± .06 | .79 ± .04 | .88 ± .02 | .85 ± .03 |
| Cowpea, lupine[c] | .335± .057 | .570± .042 | .675± .035 | .785± .025 | .775± .025 |
| Alfalfa[d] | .290± .035 | .430± .030 | .495± .029 | .700± .019 | .705± .019 |
| Amaranthus | .04 ± .10 | .40 ± .09 | .45 ± .08 | .60 ± .07 | .56 ± .06 |

| | Mean. | σ |
|---|---|---|
| Evaporation (shallow tank) (kilograms per square meter) | 9.70 | 2.76 |
| Integrated radiation (calories per square centimeter) | 753 | 134 |
| Air temperature, integrated mean (degrees Centigrade) | 20.10 | 3.48 |
| Integrated wet-bulb depression (hour degrees, Centigrade) | 143 | 58 |
| Wind velocity (miles per hour) | 5.54 | 2.24 |

[a] Averages of six similar correlations involving Kubanka and Galgalus wheat, Swedish Select and Burt oats, Hannchen barley, and spring rye. The last, having on the whole the largest correlations, is also given separately.

[b] Averages of four correlations, Minnesota Amber and Dakota Amber sorghum and Kursh and Siberian Millet. These correlations were all very similar.

[c] Average of the similar correlations for cowpeas and lupine.

[d] Average of four tests with alfalfa.

[e] Published as +0.80, which seems too large. Recalculation gives +0.52.

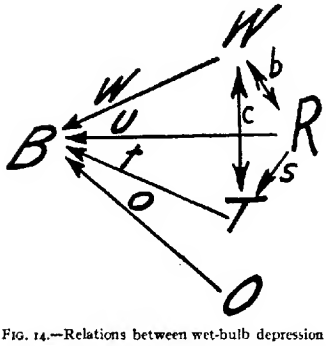


FIG. 14.—Relations between wet-bulb depression (*B*), wind velocity (*W*), radiation (*R*), and temperature (*T*) as assumed for direct analysis.

It will be interesting first to compare the direct and indirect methods of calculating path coefficients and coefficients of determination. Let us consider the relations of wet-bulb depression (*B*) to temperature (*T*), wind velocity (*W*), and radiation (*R*). Since the direct methods are only applicable in systems in which each variable is connected with every other variable, the diagram of relations is as shown in figure 14. Outstanding factors, independent of *W*, *R*, and *T* are represented by *O*.

INDIRECT METHOD

Six equations can be formed, expressing the six known correlations in terms of the unknown path coefficients. A seventh equation represents the complete determination of $B$ by $W$, $R$, $T$, and $O$.

(1) $r_{BW} = 0.28 = w + t(c+bs) + ub.$
(2) $r_{BR} = .48 = wb + ts + u.$
(3) $r_{BT} = .59 = w(c+bs) + t + us.$
(4) $r_{WR} = -.01 = b.$
(5) $r_{WT} = -.02 = c + bs.$
(6) $r_{RT} = .47 = s.$
(7) $o^2 + w^2 + t^2 + u^2 + 2wt(c+bs) + 2wub + 2uts = 1.$

The values of $b$ and $s$ are given directly from equations (4) and (6), and the value of $c$ ($= -0.0153$) can then be obtained from (5). The solution of (1), (2), and (3) gives $w = 0.2921$, $t = 0.4735$, and $u = 0.2604$. Finally, from (7) we obtain $o^2 = 0.5138$ as the degree of determination by outstanding factors.

| | | | |
|---|---|---|---|
| $d_{B\cdot O} = o^2$ | = | 0.5138 | |
| $d_{B\cdot W} = w^2$ | = | .0853 | $p_{B\cdot W} = w = 0.2921$ |
| $d_{B\cdot T} = t^2$ | = | .2242 | $p_{B\cdot T} = t =$ .4735 |
| $d_{B\cdot R} = u^2$ | = | .0678 | $p_{B\cdot R} = u =$ .2604 |
| $d_{B\cdot \overline{WT}} = 2wt(c+bs)$ | = | −.0055 | |
| $d_{B\cdot \overline{WR}} = 2wub$ | = | −.0015 | |
| $d_{B\cdot \overline{RT}} = 2uts$ | = | .1159 | |
| | | 1.0000 | |

DIRECT METHODS

According to the formulae given in part I we have—

$$d_{B\cdot O} = \frac{\phi(BWRT)}{\phi(WRT)}$$

$$d_{B\cdot W} = \frac{\phi(BRT) - d_{B\cdot O}\phi(RT)}{\phi(WRT)}$$

$$d_{B\cdot R} = \frac{\phi(BWT) - d_{B\cdot O}\phi(WT)}{\phi(WRT)}$$

$$d_{B\cdot T} = \frac{\phi(BRW) - d_{B\cdot O}\phi(RW)}{\phi(WRT)}$$

where

$$\begin{aligned}\phi(BWRT) = 1 &- r^2_{BW} + 2r_{BW}r_{WR}r_{RB} - 2r_{BW}r_{WR}r_{RT}r_{TB} + r^2_{BW}r^2_{RT} \\ &- r^2_{BR} + 2r_{BW}r_{WT}r_{TB} - 2r_{BW}r_{WT}r_{TR}r_{RB} + r^2_{BR}r^2_{WT} \\ &- r^2_{BT} + 2r_{BR}r_{RT}r_{TB} - 2r_{BR}r_{RW}r_{WT}r_{TB} + r^2_{BT}r^2_{WR} \\ &- r^2_{WR} + 2r_{WR}r_{RT}r_{TW} \\ &- r^2_{WT} \\ &- r^2_{RT}\end{aligned}$$

$\phi(WRT) = 1 - r^2_{WR} - r^2_{WT} - r^2_{RT} + 2r_{WR}r_{RT}r_{TW}$
$\phi(BWR)$, etc., are analogous to $\phi(WRT)$
$\phi(RT) = 1 - r^2_{TR}$ $\phi(WT)$, etc., are analogous to $\phi(RT)$.

By substitution of the correlations in these formulae the following results are obtained:

$$\phi(BWRT) = 0.4002$$

| | | | |
|---|---|---|---|
| $\phi(BWR) = .6884$ | $\phi(BW) = 0.9216$ | $\phi(WR) = 0.9999$ |
| $\phi(BWT) = .5665$ | $\phi(BR) = .7696$ | $\phi(WT) = .9996$ |
| $\phi(BRT) = .4668$ | $\phi(BT) = .6519$ | $\phi(RT) = .7791$ |
| $\phi(WRT) = .7788$ | | |

These give values of the coefficients of determination identical with those given by the indirect method.

This method, as was shown in part I, is essentially the same as Pearson's method of calculating multiple regression.

$$\text{Let } \Delta = \begin{vmatrix} 1 & r_{BR} & r_{BT} & r_{BW} \\ r_{RB} & 1 & r_{RT} & r_{RW} \\ r_{TB} & r_{TR} & 1 & r_{TW} \\ r_{WB} & r_{WR} & r_{WT} & 1 \end{vmatrix} = \begin{vmatrix} 1 & 0.48 & 0.59 & 0.28 \\ .48 & 1 & .47 & -.01 \\ .59 & .47 & 1 & -.02 \\ .28 & -.01 & -.02 & 1 \end{vmatrix} = 0.4002$$

Let $\Delta_{BB} = \Delta$ with column B, row B, deleted.

$\Delta_{BB} = 0.7788$, $\Delta_{BR} = 0.2028$, $\Delta_{BT} = 0.3687$, $\Delta_{BW} = 0.2275$

$$p_{B\cdot W} = \frac{\Delta_{BW}}{\Delta_{BB}} = 0.2921 \qquad d_{B\cdot O} = \frac{\Delta}{\Delta_{BB}} = 0.5139$$

$$p_{B\cdot R} = \frac{\Delta_{BR}}{\Delta_{BB}} = 0.2604$$

$$p_{B\cdot T} = \frac{\Delta_{BT}}{\Delta_{BB}} = 0.4735.$$

These values are identical with those obtained by the preceding methods.

It will be seen that the first method, while apparently less direct than the others, is really less laborious. The solution of three simultaneous equations requires merely the evaluation of a determinant of the third order instead of one of the fourth order, as in the last method. The expression $\phi(BWRT)$ in the second method is, of course, merely an expansion of the same determinant of the fourth order as that used in the last. The indirect method, moreover, gives more insight into the processes followed than the others in which there is a substitution in what appear to be arbitrary formulae. In line with this last point, the indirect method is more flexible in that it can be used to test out the consequences of any assumed relation among the factors.

## ANALYSIS OF CAUSAL RELATIONS

In attempting to interpret the present results in terms of causation, we see at once that the scheme of relations chosen is not a very satisfactory one. The wet-bulb depression was measured under shelter. Consequently the coefficient of determination, $d_{B,R} = 0.0678$, can not measure

the degree of direct determination by radiation, but determination by some factor other than wind or temperature with which radiation is correlated.

One should not attempt to apply in general a causal interpretation to solutions by the direct methods. In these cases, determination can usually be used only in the sense in which it can be said that knowledge of the effect determines the probable value of the cause. This is the sense in which Pearson's formula for multiple regression must be interpreted. If $W'$, $T'$, and $R'$ are given deviations of wind, temperature, and radiation from their mean values, the most probable value of the wet-bulb depression, $B'$, is given by the following formula:

$$\frac{B'}{\sigma_B} = \frac{W'}{\sigma_W} p_{B\cdot W} + \frac{R'}{\sigma_R} p_{B\cdot R} + \frac{T'}{\sigma_T} p_{B\cdot T}.$$

This formula can only be used for conditions which are similar to those for which the values of the path coefficients were calculated. If path coefficients were calculated in a system which truly represented the causal relations, the formula would give the value of the wet-bulb depression under any set of conditions in so far as it is determined by the factors considered.

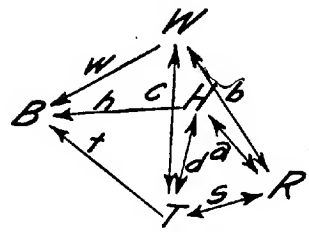


FIG. 15.—Relations between factors of figure 14 and absolute humidity ($H$) expressing causal relations better than figure 14 but adapted only to indirect analysis.

The causal factors which actually determine wet-bulb depression are temperature, absolute humidity ($H$), and wind velocity (fig. 15). Radiation can be introduced into the scheme as a factor correlated with these causal factors. Wind velocity is correlated to such a very slight extent with temperature and radiation that its correlation with absolute humidity can probably be neglected without serious error. The relations between radiation, temperature, and absolute humidity are undoubtedly very complex. Radiation has a direct positive influence on temperature. Both radiation and temperature have positive effects on absolute humidity by increasing evaporation. Correlation between absolute humidity and temperature would be expected, because with reduced temperature the saturation point is reached at a lower absolute humidity and the excess moisture is precipitated. Increase in humidity, on the other hand, tends to reduce the radiation which reaches the earth, and directly or indirectly this has a negative influence on all three of the correlations.

There are not enough data to estimate the importance of all of these paths of influence. Even if we represent the complex of paths connecting $H$, $R$, and $T$ merely by three correlations, the diagram has eight paths to solve. The six correlations between $B$, $W$, $R$, and $T$ and the statement

in regard to complete determination of $B$ by $W$, $H$, and $T$ furnish only seven equations.

Fortunately, data are given in another paper by Briggs and Shantz (3) from which an eighth equation can be derived. In this paper the average value of each of the measured factors is given for each hour of the day. The cycle of changes in wet-bulb depression follows very closely the changes in temperature. In fact, there should be very little, if any, regular hourly cycle of changes in absolute humidity, so that the wet-bulb depression should be wholly determined by the temperature changes except for some influence of wind velocity.

Let $p_{B.T}=t$ be the path coefficient which measures the relative influence of temperature on wet-bulb depression in the variations from day to day. Let $p_{B.H}=h$, $p_{B.W}=w$, and let $\sigma_T$, $\sigma_H$, $\sigma_W$, and $\sigma_B$ be the standard deviations of the daily differences in the various factors and in wet-bulb depression. Let $T'-T''$, etc., be the actual differences in temperature, etc., at certain times. The difference to be expected in wet-bulb depression, $B'-B''$, is as follows:

$$\frac{B'-B''}{\sigma_B}=\frac{T'-T''}{\sigma_T}t+\frac{W'-W''}{\sigma_W}w+\frac{H'-H''}{\sigma_H}h.$$

While $t$, $w$, and $h$ are assumed to measure the relative influence of temperature, wind, and humidity in the variations from day to day, the foregoing formula should apply under any conditions, if $t$, $w$, and $h$ were calculated from a system which represented truly causal relations. The expression $\frac{\sigma_B}{\sigma_T}t$ is shown in part I to give the change in wet-bulb depression ($B$) directly caused by a unit change in temperature. The relative importance of the various factors in determining the variations from hour to hour is very different from that from day to day, but the change in wet-bulb depression caused by unit changes in temperature, wind velocity, or absolute humidity should always be the same so long as the relations are substantially linear.

The greatest difference in temperature within an average day in the data was between 5 a. m. and 3 p. m. This is given as 32.7° F., or 18.167° C. The difference in wet-bulb depression between these hours was 21.8° F., or 12.111° C. The difference in average wind velocity was 2.5 miles per hour. The standard deviations of the daily variations have already been given. $\sigma_T=3.48$ day degrees C., $\sigma_B=58$ hour degrees C. integrated for 24 hours. This means 2.4167 degrees C. $\sigma_W=2.24$ miles per hour. We will assume that there is no difference in absolute humidity ($H'-H''=0$). Substituting those values in the formula for wet-bulb depression, we get

$$\frac{12.111}{2.4167}=\frac{18.167}{3.48}t+\frac{2.50}{2.24}w$$

$$5.0114=5.2204t+1.1161w.$$

We now have eight equations from which to find eight unknown path coefficients.

(1) $r_{BW} = 0.28 = w + tc.$
(2) $r_{BR} = .48 = ts + bw + ah.$
(3) $r_{BT} = .59 = t + dh + wc.$
(4) $r_{WR} = -.01 = b.$
(5) $r_{WT} = -.02 = c.$
(6) $r_{RT} = .47 = s.$
(7) $w^2 + h^2 + t^2 + 2wtc + 2htd = 1.$
(8) $5.0114 = 5.2204t + 1.1161w.$

Equations (4), (5), and (6) give $b$, $c$, and $s$ directly. Solution of (1) and (8) gives $t = 0.8963$, $w = 0.2979$.

From (2) $ah = 0.0617$
From (7) $h^2 = .6570$, $h = -0.8105$, $a = -0.0761$
From (3) $dh = -.3003$, $d = .3706$
$r_{BH} = h + td = -0.4784.$

The coefficients of determination, the path coefficients, and the correlations are thus as follows:

| | | |
|---|---|---|
| $d_{B\cdot T} = 0.8034$ | $p_{B\cdot T} = 0.8963$ | $r_{BT} = 0.5900$ |
| $d_{B\cdot H} = .6570$ | $p_{B\cdot H} = -.8105$ | $r_{BH} = -.4784$ |
| $d_{B\cdot W} = .0888$ | $p_{B\cdot W} = .2979$ | $r_{BW} = .2800$ |
| $d_{B\cdot \overline{HT}} = -.5384$ | | |
| $d_{B\cdot \overline{WT}} = -.0107$ | | $r_{HR} = -.0761$ |
| 1.0001 | | $r_{HT} = .3706$ |
| | | $r_{RT} = .4700.$ |

It turns out that the differences between different days in wet-bulb depressions are due to a somewhat greater extent to differences in temperature (0.80) than to absolute humidity (0.66). The variation in wet-bulb depression would be much greater were it not that these factors vary together but act on wet-bulb depression in opposite directions and so tend to balance each other ($d_{B\cdot\overline{HT}} = 0.54$). Temperature shows a rather strong positive correlation with absolute humidity (0.37) as well as with radiation (0.47), but the various paths of influence between radiation and absolute humidity almost balance each other ($r_{HR} = -0.08$).

These results can now be used in finding the relative importance of the various factors which determine evaporation or transpiration. In figure 16, $X$ may represent either evaporation or the transpiration of any plant. Radiation must be considered as a direct causal factor in these cases.

The following four equations can be made with which to solve the path coefficients from $W$, $H$, $R$, and $T$ to $X$:

$$\begin{aligned} r_{XW} &= w' + t'c + u'b \\ r_{XT} &= w'c + t' + u's + h'd \\ r_{XR} &= w'b + t's + u' + h'a \\ r_{XB} &= w'r_{BW} + t'r_{BT} + u'r_{BR} + h'r_{BH}. \end{aligned}$$

Substituting the values already found for $a$, $b$, $c$, $d$, $w$, $h$, $t$, and $r_{BR}$, we have

$$\begin{aligned} r_{XW} &= +1.00w' - 0.02t' - 0.01u' \\ r_{XT} &= -\ .02w' + 1.00t' + \ .47u' + 0.3706h' \\ r_{XR} &= -\ .01w' + \ .47t' + 1.00u' - \ .0761h' \\ r_{XB} &= +\ .28w' + \ .59t' + \ .48u' - \ .4784h'. \end{aligned}$$

The solution is as follows:

$$\begin{aligned} w' = p_{X\cdot W} &= +0.9971r_{XW} + 0.0143r_{XT} - 0.0022r_{XR} + 0.0114r_{XB} \\ t' = p_{X\cdot T} &= -\ .2207r_{XW} + \ .8943r_{XT} - \ .8175r_{XR} + \ .8228r_{XB} \\ u' = p_{X\cdot R} &= +\ .1488r_{XW} - \ .3633r_{XT} + 1.4155r_{XR} - \ .5067r_{XB} \\ h' = p_{X\cdot H} &= +\ .4607r_{XW} + \ .7468r_{XT} + \ .4107r_{XR} - 1.5772r_{XB}. \end{aligned}$$

It is merely necessary to substitute the values of the correlations of evaporation or transpiration with wind velocity, temperature, radiation, and wet-bulb depression, as given in Table I, to find the four path coefficients in each case. The results are given in Table II. These have all been checked by substitution in the fourth equation ($r_{XB} = +0.28w' + 0.59t' + 0.48u' - 0.4784h'$). The correlation between evaporation and the transpiration of any plant can be deduced from the formula $r_{XE} = w'r_{EW} + t'r_{ET} + u'r_{ER} + h'r_{EH}$. The correlations of evaporation with wind velocity, temperature, and radiation have been given in Table I as 0.38, 0.56, and 0.68, and that with humidity can be calculated by the formula $r_{EH} = p_{E\cdot H} + a p_{E\cdot R} + d p_{E\cdot T} = -0.2651$. Thus $r_{XE} = 0.38w' + 0.56t' + 0.68u' - 0.2651h'$. The calculated results in column 6 of Table II are compared with actual correlations between evaporation and transpiration in column 7. The correlation of evaporation with itself comes out 0.839 by this formula. There should, however, be an additional term ($p_{X\cdot O}r_{EO}$) in the formula to allow for correlation through other factors ($O$) than $W$, $T$, $R$, and $H$. From Table III we find that evaporation is determined

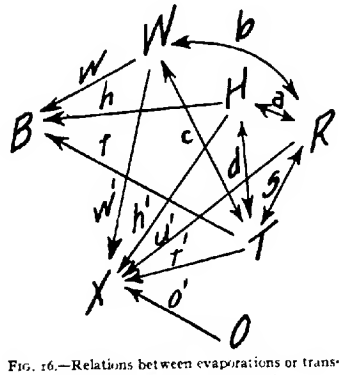


FIG. 16.—Relations between evaporations or transpiration (X) and the system shown in figure 15.

to a considerable extent ($d_{E\cdot O}=0.161$) by outstanding factors. The additional term in this case would have this value and when added to 0.839 gives 1, as it should. With one exception, the calculated correlation between transpiration and evaporation is a little smaller than the actual correlation. This means either that there is some additional factor which should be allowed for or else that the path coefficients with $W$, $T$, $R$, and $H$ are not given quite their due weight, owing perhaps to lack of complete linearity in the correlations.

TABLE II.—*Table of calculated path coefficients*

| | Wind. | Temperature. | Radiation. | Absolute humidity. | Correlation with evaporation. | |
|---|---|---|---|---|---|---|
| | $p_{X\cdot W}$ | $p_{X\cdot T}$ | $p_{X\cdot R}$ | $p_{X\cdot H}$ | Calculated. $r_{XE}$ | Actual. $r_{XE}$ |
| Wet-bulb depression | 0.298 | 0.896 | 0 | −0.811 | 0.830 | 0.83 |
| Evaporation (shallow tank) | .395 | .544 | .395 | −.437 | (.839) | 1.00 |
| Transpiration: | | | | | | |
| Small grains | .238 | .779 | .249 | −.489 | .826 | .87 |
| Rye | .209 | .853 | .207 | −.583 | .852 | .91 |
| Sorghum and millet | .234 | .718 | .203 | −.421 | .741 | .713 |
| Sudan grass (inclosure) | .539 | .870 | .230 | −.216 | .838 | .93 |
| Sudan grass (open) | .339 | .928 | .059 | −.375 | .788 | .82 |
| Dent corn | .297 | .815 | .109 | −.405 | .751 | .79 |
| Algerian corn | .349 | .851 | .194 | −.391 | .844 | .85 |
| Cowpea and lupine | .351 | .710 | .274 | −.346 | .768 | .775 |
| Alfalfa | .303 | .603 | .117 | −.424 | .645 | .705 |
| Amaranthus | .052 | .560 | .105 | −.428 | .518 | .560 |
| Average transpiration | .279 | .733 | .181 | −.420 | .751 | .781 |

TABLE III.—*Coefficients of determination*

| | Wind. | Temperature. | Radiation. | Absolute humidity. | Joint determination. | | | | | Residual. |
|---|---|---|---|---|---|---|---|---|---|---|
| | $d_{X\cdot W}$ | $d_{X\cdot T}$ | $d_{X\cdot R}$ | $d_{X\cdot H}$ | $d_{X\cdot WT}$ | $d_{X\cdot WR}$ | $d_{X\cdot TR}$ | $d_{X\cdot TH}$ | $d_{X\cdot RH}$ | $d_{X\cdot O}$ |
| Wet-bulb depression | 0.089 | 0.803 | 0 | 0.657 | −0.011 | 0 | 0 | −0.538 | 0 | 0 |
| Evaporation | .156 | .299 | 0.156 | .191 | − .009 | − .003 | 0.202 | − .176 | +0.026 | 0.161 |
| Transpiration: | | | | | | | | | | |
| Small grain | .057 | .607 | .062 | .240 | − .007 | − .001 | .182 | − .283 | + .019 | .125 |
| Rye | .044 | .728 | .043 | .340 | − .007 | − .001 | .166 | − .369 | + .018 | .038 |
| Sorghum and millet | .055 | .516 | .047 | .177 | − .007 | − .001 | .137 | − .224 | + .013 | .293 |
| Sudan (inclosure) | .290 | .757 | .017 | .047 | − .019 | − .001 | .106 | − .140 | + .004 | (− .062) |
| Sudan (open) | .115 | .861 | .003 | .141 | − .013 | − .000 | .081 | − .258 | + .003 | .096 |
| Dent corn | .088 | .564 | .012 | .164 | − .010 | − .001 | .084 | − .244 | + .007 | .237 |
| Algerian corn | .122 | .724 | .038 | .153 | − .012 | − .001 | .155 | − .247 | + .012 | .057 |
| Cowpea and lupine | .123 | .504 | .046 | .120 | − .010 | − .002 | .143 | − .182 | + .011 | .47 |
| Alfalfa | .092 | .364 | .014 | .180 | − .007 | − .001 | .067 | − .190 | + .008 | .474 |
| Amaranthus | .003 | .314 | .011 | .183 | − .001 | − .000 | .055 | − .178 | + .007 | .607 |
| Average transpiration | .078 | .537 | .033 | .176 | − .008 | − .001 | .124 | − .228 | + .012 | .277 |

The coefficients of determination are given in Table III. The difference between their sum and unity is given in the last column as $d_{X\cdot O}$, the determination by outstanding factors. As suggested above, the assumption that all the fundamental correlations are linear may involve

some error which would tend to underweight the coefficients of determination between transpiration and the known factors and so overweight the apparent degree of determination by outstanding factors. In certain cases, however, the residue is so small, in one case actually coming out negative, that it is probable that this is not an important source of error. The residual determination is greatest for the crops which were cut twice during the season—namely alfalfa and amaranthus. There were considerable periods following each cutting during which the absolute value of the transpiration was small.

Wind velocity has about the same relative value as a factor in determining transpiration as it has in determining wet-bulb depression. Its relative importance is a little greater for determining evaporation from the shallow tank.

Temperature is somewhat more important than absolute humidity in determining the variations in wet-bulb depression and rate of evaporation from day to day. It is very much the most important factor in determining the rate of transpiration in all the plants.

Radiation is an important factor in evaporation, coming out equal to wind velocity and only slightly less important than absolute humidity. In the plants, on the other hand, it is almost a negligible factor.

Comparing transpiration in the average plant with evaporation in the sun from a shallow tank, we find that the former is influenced relatively much more by temperature, to about the same degree by absolute humidity, somewhat less by wind velocity, and very much less by radiation. The four factors are much more nearly equal in importance in the case of evaporation ($d_{E\cdot T}=0.30$, $d_{E\cdot H}=0.19$, $d_{E\cdot W}=0.16$, $d_{E\cdot R}=0.16$) than in the case of transpiration ($d_{X\cdot T}=0.55$, $d_{X\cdot H}=0.18$, $d_{X\cdot W}=0.09$, $d_{X\cdot R}=0.04$). In comparing the importance of these factors it should be added that radiation has an importance somewhat in excess of its direct influence, in that its variations are correlated with those of temperature. Humidity has reduced importance, since, though correlated with temperature, it affects evaporation and transpiration in the opposite direction.

## OTHER APPLICATIONS

The method of analysis presented here can readily be applied to the problem of the relative importance of heredity and environment. An application of this kind to the case of the piebald pattern of guinea pigs has already been published (9), and one to the resistance of the same animal to tuberculosis is in press.[1] The method can be applied also to such a problem as the determination of the effects of various systems of mating, such as inbreeding, line breeding, and assortative mating on the genetic composition of an originally random-bred stock.[2]

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# MEASUREMENT OF THE AMOUNT OF WATER THAT SEEDS CAUSE TO BECOME UNFREE AND THEIR WATER-SOLUBLE MATERIAL

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## INTRODUCTION

It has been shown that soils cause water to become inactive or unfree, as is indicated by its refusal to freeze or to function as a solvent. The magnitude of this unfree water has been measured by means of the dilatometer method,[1] which has proved most convenient, appropriate, and unique for this purpose. The principle of this method is based upon the fact that water expands upon freezing. If the amount of expansion that a certain amount of water (1 gm.) produces upon freezing is known, then the quantity of water that freezes in the soil can be calculated from the magnitude of expansion produced. On the basis of this dilatometer method the water in the soil has been classified anew as follows:

1. Gravitational water, unsuitable to plants.
2. Free water, readily available to plants.
3. Unfree water { Capillary, adsorbed, very slightly available to plants. / Combined { water of hydration / water of solid solution } very unavailable to plants.

The free water is that which freezes very readily at the supercooling of $-1.5°$ C.; the capillary, adsorbed water is that which freezes from the temperature of $-1.5°$ to $-78°$; while the combined water is that which does not freeze at all, even at the extreme temperature of $-78°$.

## AMOUNT OF WATER THAT SEEDS CAUSE TO BECOME UNFREE

It is, of course, very well known that seeds absorb large quantities of water and with a considerable force. Seeds like the lima bean, cowpea, soybean, clover, and alfalfa absorb over 100 per cent of their dry weight of water; while seeds like the wheat, rye, and corn absorb about 50 per cent of their dry weight of water. The great attraction that seeds have for water is best realized by the fact that they will abstract the moisture from the soils even down to the point of air-dryness. Whitney

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and Cameron [1] found, for instance, that when 50 gm. of seeds of cowpeas were mixed with 50 gm. of soil containing 15 per cent of water, the seeds had in 12 hours gained 12.1 per cent of water and had left in the soil only 1.3 per cent—that is, the soil was reduced practically to air-dry condition. It appears, therefore, that the power of seeds to absorb water is very much greater than that of soils. Some attempts have been made to measure the magnitude of the initial attraction that seeds possess for water. Especially notable is the work in this direction of Shull [2] who attempted to measure the attraction of seeds of Xanthium for water, and then he used these seeds to measure in turn the moisture-holding forces of soils. Shull found that the air-dry seeds of Xanthium show an initial attraction for water of nearly 1,000 atmospheres.

Since it was found that soils cause water to become unfree, the extent varying with the character of the soil, the question arose whether the seeds also cause water to become unfree, and if so, to what extent. It was reasoned and anticipated that since seeds possess a greater attraction for water as evidenced by their power to abstract moisture from the soil itself even down to the point of dryness, they ought to cause a larger amount of water to become unfree.

In order to obtain information bearing upon these questions a general investigation of the problem was undertaken. The type of dilatometer used and the general procedure followed were the same as those used in the study of soils. The procedure consisted in weighing out carefully about 10 gm. of air-dry seeds and placing them in water to soak for about two days. Then they were taken out, pressed between filter papers in order to eliminate their excess of water, weighed again quickly, and introduced into the dilatometer. The unoccupied space in the dilatometer was then filled with ligroin, and care was taken to expel all the air. The mouth of the dilatometer was then carefully stoppered, and the contents were placed to cool in a temperature of $-3°$ C. When this temperature was attained by the contents, as indicated by the column of ligroin in the stem, which remained stationary, the water in the seeds was caused to freeze. This was accomplished by taking hold of the dilatometer by the stem and moving it gently in the cooling mixture until solidification began, which was indicated by the rise of the ligroin in the stem. The dilatometer was allowed to remain in the cooling mixture with frequent movements until the rise of the ligroin in the stem ceased. The total rise of the ligroin in the stem was taken to represent the total amount of expansion due to the formation of ice.

In order to determine the effect of repeated freezing or of lower temperature upon the amount of water that seeds cause to become unfree, the

[1] WHITNEY, Milton, and CAMERON, F. K. INVESTIGATIONS IN SOIL FERTILITY. U. S. Dept. Agr. Bur. Soils Bul. 23, p. 50. 1904.

[2] SHULL, Charles Albert. MEASUREMENT OF THE SURFACE FORCES IN SOILS. *In* Bot. Gaz. v. 62, no. 1, p. 1-31, 5 fig. 1916. Literature cited, p. 29-31.

seeds in the dilatometer were thawed and refrozen either at the temperature of $-3°$ or of $-20°$ C. In the latter case, the contents of the dilatometer were allowed first to supercool at $-3°$ and to assume equilibrium at this temperature; then they were put in the temperature of $-20°$, allowed to remain there for about one hour, and were then placed back into the temperature of $-3°$ and allowed to attain equilibrium.

In all, 14 different kinds of seeds were used. These were spring wheat, winter wheat, barley, rye, white corn, yellow corn, broom corn, alfalfa, alsike clover, mammoth clover, cowpeas, field peas, field white beans, and black soybeans.

In Table I are presented part of the data obtained. They show the amount of water the different kinds of seeds absorbed and the quantity they caused to become unfree, as indicated by its refusal to freeze for the first time at the temperature of $-3°$ C. The quantity of unfree water is expressed both in cubic centimeters and in percentage based on the weight of the air-dry seeds. The factor used for converting the volume of expansion due to the ice formation into the corresponding weight of water was that obtained experimentally and used in the study of the soil—namely, 1 cc. of water expands approximately 0.1 cc. upon freezing.

TABLE I.—*Amount of water that failed to freeze in seeds when they were supercooled and frozen for the first time in a temperature of $-3°$ C.*

| Kind of seeds. | Weight of air-dry seeds. | Weight of water-soaked seeds. | Absorbed water which failed to freeze. | |
|---|---|---|---|---|
| | *Gm.* | *Gm.* | *Cc.* | *Per cent.* |
| Spring wheat | 11.210 | 18.290 | 2.880 | 25.70 |
| Winter wheat | 11.250 | 18.510 | 3.360 | 30.10 |
| Barley | 10.770 | 18.080 | 4.310 | 40.02 |
| Rye | 10.230 | 18.150 | 3.920 | 40.20 |
| White corn | 12.075 | 17.640 | 3.765 | 31.18 |
| Yellow corn | 12.215 | 17.265 | 4.650 | 38.09 |
| Brown corn | 10.020 | 16.230 | 2.510 | 25.05 |
| Alfalfa | 11.300 | 25.730 | 8.430 | 74.60 |
| Alsike clover | 11.200 | 24.200 | 7.400 | 66.08 |
| Mammoth clover | 11.200 | 25.700 | 7.100 | 63.40 |
| Cowpeas | 9.210 | 20.790 | 6.680 | 72.54 |
| Field peas | 10.070 | 20.800 | 7.730 | 76.76 |
| Field white peas | 10.275 | 20.320 | 5.445 | 52.96 |
| Black soybeans | 7.110 | 16.920 | 5.310 | 74.68 |

From the foregoing experimental data it is at once seen that the amount of water which the seeds cause to become unfree is really very great in nearly all the different kinds of seeds. It varies from about 25.05 per cent with broom corn to 76.76 per cent with black soybeans. It appears that the alfalfa, clover, cowpeas, and bean seeds cause a considerably larger amount of water to become unfree than the wheat, rye, barley, and corn seeds. As has already been mentioned, this

percentage of unfree water is based only on the absorbed water; the hygroscopic moisture is not included in it. Hence the total amount of unfree water in the seeds is still greater than is represented by these numerical data.

In the foregoing investigation the seeds were supercooled and frozen only once in the temperature of −3° C. The investigations with soils revealed the fact that repeated freezing and thawing and lower temperature tended to reduce the amount of unfree water in soils, especially in the fine-textured and colloidal soils. In order to ascertain whether repeated freezing and thawing and lower temperature brought also a diminution in the unfree water in the seeds, the latter were frozen and thawed three times in a temperature of −20°. Finally they were supercooled to −3°, frozen in −20° for one hour, and brought back again to −3°, where the total expansion was measured. Table II contains the results obtained from this investigation. For immediate and convenient comparison the results obtained at the first freezing are also presented in this table.

TABLE II.—*Effect of repeated freezing and thawing and low temperature on the amount of water that fails to freeze in seeds*

| Kind of seeds. | Water which failed to freeze, (frozen only once). | Water which failed to freeze, (frozen and thawed four times). | Difference in favor of seeds frozen only once. |
|---|---|---|---|
| | *Per cent.* | *Per cent.* | *Per cent.* |
| Spring wheat | 25. 70 | 25. 70 | 0. 00 |
| Winter wheat | 30. 10 | 28. 98 | 1. 12 |
| Barley | 40. 02 | 35. 38 | 4. 64 |
| Rye | 40. 20 | 35. 39 | 4. 81 |
| White corn | 31. 18 | 23. 70 | 7. 48 |
| Yellow corn | 38. 00 | 26. 62 | 11. 47 |
| Broom corn | 25. 05 | 13. 08 | 11. 97 |
| Alfalfa | 74. 60 | 40. 98 | 33. 62 |
| Alsike clover | 66. 08 | 40. 18 | 25. 90 |
| Mammoth clover | 63. 40 | 41. 08 | 22. 32 |
| Cowpeas | 72. 54 | 39. 95 | 32. 59 |
| Field peas | 76. 76 | 57. 90 | 18. 86 |
| Field white peas | 52. 96 | 26. 78 | 26. 33 |
| Black soybeans | 74. 68 | 47. 96 | 26. 72 |

It is readily seen that repeated freezing and thawing has a very marked diminishing effect on the unfree water in the seeds, especially with certain kinds of seeds. In those seeds which contained a tremendous amount of unfree water at the first freezing, such as the alfalfa, clover, peas, and beans, the diminution in the quantity of unfree water by repeated freezing and thawing is very considerable, amounting in some cases to over 33 per cent. On the other hand, in such seeds as the

wheat, corn, barley, and rye the process of repeated freezing and thawing had very little effect if any on the unfree water.

The process of repeated freezing and thawing, therefore, has practically the same influence in seeds as it has in soils. In both cases it tends to diminish the amount of unfree water in some seeds or soils more than in others.

In explaining the decrease of the unfree water by repeated freezing and thawing two hypotheses were presented. In the one it was suggested that part of the water is held by the capillarities of the soil and does not freeze. Upon repeated freezing and thawing these capillarities are destroyed, and the water they held is liberated or becomes free and freezes readily.

In the second hypothesis it was assumed that soils such as clays, clay loams, silts, muck, and peats contained a considerable amount of colloidal material which held water in such a manner that it does not freeze. Upon repeated freezing and thawing, however, these colloids are coagulated or destroyed, and the water they held is liberated or becomes free and readily freezes.

These suggested explanations with few modifications may apply also to seeds. There is no doubt that the living tissue as well as its capillaries and colloidal material are affected or destroyed by severe freezing.

It may be of interest to record here that when very old corn seed was employed or corn seed that had been frozen in the field, no water was caused to become unfree. Apparently long age or previous freezing of the corn seed destroyed its power to cause water to become unfree. This phenomenon, however, did not appear in the other seeds.

According to the classification of moisture in the soils based on the dilatometer method, the water which freezes after the first freezing may be classified as capillary-adsorbed water, while that which refuses to freeze after the fourth freezing and at the low temperature may be classified as combined, probably in the form of water of hydration and water of solid solution.

However, the division of the unfree water into capillary adsorbed and combined water is probably not so sharp in seeds as in soils, because in the seeds there is a considerable quantity of water-soluble material which causes a high freezing-point depression, and this in turn decreases the amount of water that freezes at the degree of supercooling employed. As is well known, there is always a tendency for an equilibrium to be established between the liquid-solvent, solid-solvent, and the solute at any temperature below freezing until the cryohydric temperature is reached. Some of the water, therefore, which refused to freeze at $-20°$ C. or which froze and melted again at $-3°$ may be due to the water-soluble material of the seeds. It is believed, however, that the amount of water that was prevented from freezing by the high freezing-point depression of the seeds is probably not very great.

## AMOUNT OF WATER-SOLUBLE MATERIAL IN SEEDS AS MEASURED BY THE FREEZING-POINT METHOD

Recognizing the influence that high concentration of solution has upon the quantity of water that refuses to freeze, the authors always determined the freezing-point depression [1] of the seeds after they were used for the dilatometer measurements. It was found that the magnitude of this depression was high for most of the seeds. Since the seeds, however, used in the dilatometer measurements were allowed to stand about two days in excess of water and were then subjected to alternate freezing and thawing, it was thought that the depression values obtained were the result of the biological and physical changes that the seeds underwent. In order to ascertain, however, whether the seeds contained water-soluble material in the dry condition they were ground very fine and then portions of 10 gm. were mixed with 20 cc. of water in a freezing-point tube. The mixture was allowed to stand for about 40 minutes, and then its freezing-point depression was determined in the usual way. Table III contains the results obtained. The values of the freezing-point depression have also been calculated into osmotic pressure in atmospheres after the table of osmotic pressures worked out by Harris and Gortner.[2]

TABLE III.—*Freezing-point depression and osmotic pressure of dry seeds when 10 gm. of powdered dry seeds were mixed with 20 cc. of water*

| Kind of seeds. | Freezing-point depression. | Osmotic pressure. |
|---|---|---|
| | ° *C.* | *Atmospheres.* |
| Spring wheat | 0. 280 | 3. 375 |
| Rye | . 352 | 4. 243 |
| Buckwheat | . 280 | 3. 375 |
| White corn | . 340 | 4. 098 |
| Broom corn | . 280 | 3. 375 |
| Sorghum | . 580 | 6. 988 |
| Alfalfa | . 610 | 7. 349 |
| Alsike clover | . 650 | 7. 830 |
| Mammoth clover | . 650 | 7. 830 |
| Cowpeas | . 715 | 8. 612 |
| Field peas | . 550 | 6. 626 |
| Field white beans | . 685 | 8. 251 |
| Black soybeans | . 560 | 6. 747 |
| Speckled wax beans | 1. 180 | 13. 336 |
| Red kidney beans | 1. 060 | 12. 760 |

The results in Table III are very surprising. They show most strikingly that there is a tremendous amount of readily water-soluble material in seeds, and in some seeds much more than in others. Thus the depression varies from 0.280° C. in wheat to 1.180° in speckled wax beans. When

[1] BOUYOUCOS, George J., and MCCOOL, M. M. OP. CIT.

[2] HARRIS, J. Arthur, and GORTNER, Ross Aiken. NOTES ON THE CALCULATION OF THE OSMOTIC PRESSURE OF EXPRESSED VEGETABLE SAPS FROM THE DEPRESSION OF THE FREEZING POINT, WITH A TABLE FOR THE VALUES OF P FOR $\Delta = 0.001°$ TO $\Delta = 2.999°$. *In* Amer. Jour. Bot., v. 1, no. 2, p. 75–78. 1914.

it is considered that this relatively large depression is obtained in a ratio of 1 of seeds to 2 of water (10 gm. of seeds and 20 cc. of water), then it can be imagined what the depression must be at a very low moisture content. It really must be large. In the ratio given here it varies from 3.375 atmospheres in wheat to 13.336 in speckled wax beans. The great attraction that seeds possess for water and their ability to abstract it from soils even down to the point of air-dryness must be due, therefore, partly, if not largely, to their great osmotic pressure caused by their high content of easily water-soluble material.

No experimental work was performed to prove definitely the nature of the material in the seeds which went into solution to cause such great depression. But it appears to be largely water-soluble proteins such as albumins and probably also some of the mineral bases. It can not be starch, which is the most abundant constituent in the seeds, because that is very insoluble in water. A test showed, for instance, that 10 gm. of starch in the pure form in 20 cc. of water had a depression of only 0.025° C. Sugar, of course, which is soluble, is not supposed to be found in dry seeds. Furthermore, to give the high depression obtained, there has to be present a very large amount of sugar, because as it is well known that this class of material does not dissociate. All evidences, therefore, point to the proteins as the main class of constituents in the seeds which produced such high depressions in the freezing point when dry seeds in the powdered form were mixed with water.

## SUMMARY

Seeds cause part of the water which they absorb to become unfree, as is indicated by its refusal to freeze.

The dilatometer method is a convenient and appropriate method for measuring the magnitude of this unfree water in seeds.

The amount of water that seeds cause to become unfree is very large, varying from 25.05 per cent in broom corn to 76.76 per cent in black soybeans, based on the air-dry weight of seeds. Repeated freezing and thawing tends to diminish considerably the amount of unfree water, especially in some seeds.

Dry seeds contain a large amount of water-soluble material, as is evidenced by the high freezing-point depression. When 10-gm. portions of seed flour are mixed with 20 cc. of water and the mixture is allowed to stand for about 40 minutes or less, the freezing-point depression varies from 0.280° C. in wheat to 1.180° in speckled wax beans. At very low moisture content the magnitude of this depression must be very great. The magnitude of the osmotic pressure must also be correspondingly very great.

The great power that seeds possess to absorb water and to abstract it from the soil is partly if not largely due to their tremendous internal osmotic pressure.

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